Risk Factors for Double Primary Breast and Ovarian Cancer in Women Across the Risk Spectrum

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Abstract

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Advancements in medicine and technology have led to an increasing number of cancer survivors. The development of a second primary cancer is one of the most severe sequelae of a cancer diagnosis, particularly for cancers that lack an effective screening tool as with ovarian cancer. Breast and ovarian cancer are major causes of morbidity and mortality in women; in the U.S., breast cancer has the highest incidence in women and ovarian cancer is the most fatal of gynecological cancers. Further, these two cancers have been found to co-occur. Along with possible treatment effects of the first cancer, shared risk factors, shared genetics, and interactions between these two have been hypothesized to contribute to their co-occurrence. Research on shared risk factors for second cancers is lacking and being able to identify potentially modifiable factors associated with second primary cancer could improve clinical recommendations for cancer survivors. Therefore, this dissertation examined risk factors for the development of double primary breast and ovarian cancer (DPBOC) in three parts 1) a comprehensive review of the literature to identify studies assessing risk factors for DPBOC, 2) a case-control study assessing the association between three potentially-modifiable risk factors (oral contraceptive (OC) use, parity, and breastfeeding), and risk of second primary ovarian cancer following breast cancer (BR-OV), second primary breast cancer following ovarian cancer (OV-BR), single primary ovarian cancer (OV), and single primary breast cancer (BR), and 3) a cohort study assessing OC use, parity, and breastfeeding and risk of BR-OV, OV, and BR.

The comprehensive review identified few studies assessing epidemiologic risk factors for the development of DPBOC and most of the findings were not statistically significant. The majority of studies focused on treatment of breast cancer and risk of second primary ovarian cancer. While most of the findings on chemotherapy, radiotherapy, and Tamoxifen were heterogeneous and lacked statistical significance, hormone therapy for breast cancer may be associated with an increased risk of second primary ovarian cancer. The majority of studies on genetic risk factors for DPBOC looked at *BRCA*1/2

mutations or a crude measure of family history. Both *BRCA*1/2 and family history were consistently associated with risk of DPBOC, but studies varied on the extent of this risk due to differences in study design, exposure and outcome definition, and statistical power. No studies were identified examining DNA methylation and risk of DPBOC.

The case-control study used data from the three clinic-based sites of the Breast Cancer Family Registry (BCFR) which consisted of women from breast and ovarian cancer families. We observed an inverse association with both OC use (OR=0.38, 95% CI: 0.22, 0.60) and breastfeeding (OR=0.52, 95% CI: 0.31, 0.87) and risk of DPBOC, but a positive association with parity (≥2 full-term pregnancies: OR=5.78, 95% CI: 2.82, 14.58), regardless of diagnosis order (BR-OV or OV-BR). We found similar associations for our OV and BR outcomes as well. When we examined differences between high and average risk women (using *BRCA*1/2 mutation status and predicted lifetime risk of breast or ovarian cancer), the inverse association with OC use only remained in women at average risk while the inverse association with breastfeeding only remained in women at high risk. As the positive association with parity and all of our outcomes disagreed with our hypothesis we conducted several sensitivity analyses to explore this finding. Survivor bias may have influenced our results as we observed differences in our findings between cases diagnosed ≤2 or ≤5 years before the baseline interview (pseudo-incident) and cases diagnosed >2 or >5 years before the baseline interview (prevalent). Specifically, the inverse association with OC use and all of our outcomes, and the positive association with parity and all of our outcomes were attenuated in the pseudo-incident group.

To address concerns of selection and information bias in our case-control study, we conducted a cohort study using data from The Breast Cancer Prospective Family Study Cohort (ProF-SC). In contrast to our case-control findings, we observed a suggestive positive association between OC use and risk of BR-OV (HR=1.62, 95% CI: 0.91, 2.90) which became stronger in women at high risk, and an inverse association between having two or more full-term pregnancies compared to nulliparous and risk of BR-OV (HR=0.47, 95% CI: 0.22, 0.97) which did not vary by underlying risk of breast and ovarian cancer. However, our BR-OV results may have similarly been influenced by survivor bias as we observed

differences in our results between our pseudo-incident and prevalent BR-OV cases; the association between OC use and BR-OV only remained in the prevalent cases.

In summary, the results of this dissertation highlight the methodological challenges in the study of second primary cancers and the importance of considering survivor bias in a cohort of cancer survivors being followed for second cancers. Further, our results are suggestive of a discordant effect of OC use on first primary versus second primary ovarian cancer which should be explored in future studies.

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Chapter 1. Introduction

1.1. Breast and Ovarian Cancer Burden

In the U.S. it is estimated that 246,660 women will be diagnosed with breast cancer and 22,280 women will be diagnosed with ovarian cancer in 2016. The relative 5-year survival of breast cancer is 98.4%, 83.9%, and 23.8% when diagnosed localized, regional, and after metastasis, respectively; the corresponding relative 5-year survival for ovarian cancer is 92.1%, 73.1%, and 28.8% [1,2]. In contrast to established population-based screening methods for breast cancer, there are currently no effective population-based screening methods for ovarian cancer [3]; thus the majority of breast cancers (93%) are diagnosed localized or regional where survival is high [1], whereas the majority of ovarian cancers (>60%) are diagnosed after metastasis when survival is low and treatments are not effective [1,2].

1.2. Subtypes of Breast and Ovarian Cancer

Breast cancer is a heterogeneous disease defined by multiple characteristics, including histological (Table 1.1) and molecular (Table 1.2) subtypes, and research has revealed many differences between these tumor subtypes, including etiology, incidence, and survival [4-10]. Ductal carcinoma is the most common histologic subtype ranging from 68 to 74% of all invasive breast cancers. For the molecular subtypes, Luminal A tumors tend to be slower-growing and have better survival which is in part due to the hormone receptors being responsive to hormone therapy. Compared to Luminal A tumors, Luminal B tumors are typically higher grade and more aggressive. Her2+ tumors tend to be more aggressive as well but targeted therapies have improved the prognosis for women with this breast cancer subtype. Lastly, triple negative tumors are commonly found in pre-menopausal women and women with *BRCA*1/2 mutations. These tumors have poorer survival which is in part due to the lack of targeted treatment.

Table 1.1. Histological subtypes of breast cancer [11-13]

Subtype	Percent of invasive breast cancer cases
Ductal	68-74%
Lobular	8-13%
Ductal/Lobular	3-7%
Tubular	2-3%
Medullary	1%
Mucinous	1-3%
Comedo	2%
Inflammatory	1%
Papillary	1%

Table 1.2. Molecular subtypes of breast cancer [7,9,10]

Subtype	Percent of invasive breast cancer cases
Luminal A (ER and/or PR+, HER2-)	51-73%
Luminal B (ER and/or PR+, HER2+)	6-16%
HER2+ (ER-, PR-, HER2+)	5-8%
Triple Negative (ER-, PR-, HER2-)	12-20%

Ovarian cancer is also a heterogeneous disease characterized by histologic and molecular characteristics. There are three main categories of ovarian cancer based on where the tumors originated: surface epithelial-stromal tumors, sex cord-stromal tumors, and germ cell tumors. Epithelial ovarian cancer is the most common histology accounting for approximately 90% of all malignant ovarian cancers [14]. Within invasive epithelial ovarian cancers, serous is the most common subtype (Table 1.3). Ovarian tumors have further been classified into two main types based on their histology, clinicopathologic features, and genetics. Type 1 ovarian tumors include low-grade serous carcinomas, borderline serous tumors, low-grade endometriod, and mucinous and clear-cell carcinomas, and account for 25% of ovarian cancers and 10% of ovarian cancer deaths. Type 2 ovarian tumors include high-grade serous carcinomas, carcinosarcomas, and undifferentiated cancers, and account for 75% of ovarian cancer and 90% of ovarian cancer deaths [15,16] (discussed further in section 1.7). Type 1 tumors are thought to originate from borderline tumors and endometriosis, while Type 2 tumors are thought to originate from intraepithelial carcinomas in the fallopian tube [16]. Work by The Cancer Genome Atlas (TCGA) has identified molecular commonalities between serous ovarian cancer and basal-like breast cancer which is discussed in section 1.7.

Table 1.3. Histological subtypes of ovarian cancer [17-20]

Subtype	Percent of invasive ovarian surface epithelial cancers
Serous	44-71%
Endometriod	9-11%
Clear Cell	4-13%
Mucinous	3-6%
Transitional	1%
Mixed	6%

1.3. Breast and Ovarian Cancer in High Risk Families

The National Comprehensive Cancer Network (NCCN) uses the same criteria to identify women at high risk of breast and ovarian cancer: 1) known family mutation in breast cancer susceptibility genes, 2) ≥2 primary breast cancers in the same individual, 3) ≥2 primary breast cancers in individuals from the same family line, 4) ≥1 primary ovarian cancer in individual(s) from the same family line, 5) first or second degree relative with breast cancer ≤45 years, 6) ≥1 family member from the same family line with ≥1 breast cancer *and* ≥1 other cancer with an early onset, and 7) male breast cancer [21]. Although familial cancers make up a small percent of all breast (5%) [22] and ovarian (5-10%) [23] cases, women with a mutation in BRCA*1/2* and women with a family history of the disease are at a much greater risk than the general population. However, most women with a family history of breast or ovarian cancer do not carry mutations in BRCA*1/2* (approximately 75% for breast [24-26] and 56% for ovarian [27]); women with a family history alone confer a much higher risk of breast and ovarian cancer than the general population [24,28]. For breast cancer, having one affected first degree relative increases a woman's risk two-fold and having three or more affected first degree relatives increases a woman's risk four-fold [29]. For ovarian cancer, having one affected first degree relative increases a woman's risk three-fold and having multiple affected relatives (first or second degree) increases a woman's risk up to eleven-fold [30]. Since the majority of breast and ovarian cancers that occur in high risk families are not due to mutations in BRCA*1* and BRCA*2*, it is crucial to identify other pathways of risk, such as shared risk factors and epigenetics.

1.4. Screening for Breast and Ovarian Cancer

Breast and ovarian cancer have asymptomatic phases when screening tools would be beneficial in detecting early stage cancer that could be more successfully treated. Mammography has been an established screening tool for breast cancer with many randomized controlled trials (RCTs) showing a survival benefit. A meta-analysis was conducted of 8 RCTs and summarized the findings for breast cancer mortality by age group [31]. For women aged 39-49 years there was a non-statistically significant 12% reduced risk of breast cancer mortality (RR=0.88, 95% CI: 0.73, 1.003) which corresponds to 4 deaths prevented per 10,000 women over 10 years. For women aged 50-59 years there was a statistically significant 14% reduced risk of breast cancer mortality (RR=0.86, 95% CI: 0.68, 0.97) which corresponds to 5 to 8 deaths prevented per 10,000 women over 10 years. For women aged 60-69 years there was a statistically significant 33% reduced risk of breast cancer mortality (RR=0.67, 95% CI: 0.54, 0.83) which corresponds to 12 to 21 deaths prevented per 10,000 women over 10 years. For women aged 70-74 years, results were not statistically significant but the sample size was limited by low events. While mammography is generally accepted as a beneficial screening tool for breast cancer, many aspects of it are still being debated, such as optimal ages to begin and end screening, optimal screening interval, balancing the benefits with potential harms of screening, and how these factors are considered based on individual risk [31].

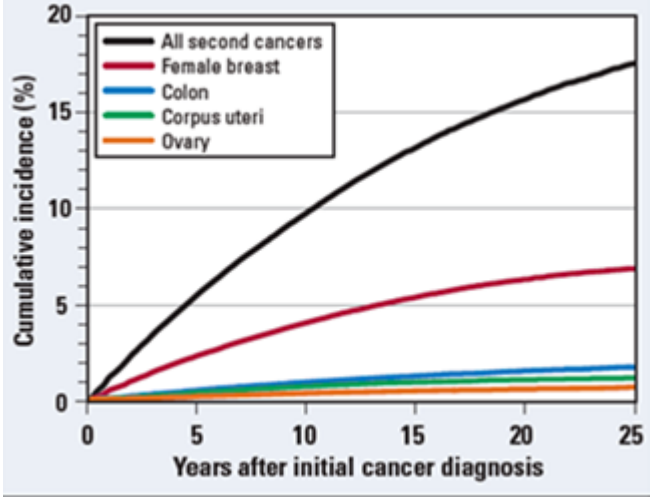
There is currently no established screening tool for ovarian cancer. The Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial was established to evaluate potential screening tools for these four cancers. For ovarian cancer, the PLCO trial evaluated the efficacy of transvaginal ultrasound and serum cancer antigen 125 (CA-125) as screening tools to reduce ovarian cancer mortality. They found no reduction in ovarian cancer mortality in the screening group receiving transvaginal ultrasound and CA-125 testing compared to the usual care group (RR=1.21, 95% CI: 0.99, 1.48) [3]. In contrast, the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS), found statistically significant reduced ovarian cancer mortality in years 7 to 14 in women screened annually with CA-125 compared to no screening when they excluded prevalent cases using the risk of ovarian cancer algorithm (ROCA) which considers both past and current CA-125 levels [32]. However, investigators re-examined data from the PLCO trial using ROCA, and still observed no reduction in ovarian cancer mortality in the screening group [33]. Given these contradictory results, continued follow-up and replication will be

needed to establish the efficacy and cost-effectiveness of CA-125 as a universal screening tool for ovarian cancer. As ovarian cancer is asymptomatic until late stages when treatment is less effective and mortality is high, establishing an effective screening tool is critical.

1.5. Double Primary Breast and Ovarian Cancer

Women who have breast cancer have an increased risk of developing ovarian cancer [34-39] and women with ovarian cancer have an increased risk of developing breast cancer [40,41]. Using SEER data for the time period 1973 to 2000, Curtis *et al*. report that the cumulative incidence of developing any second cancer after breast cancer was 17.6% within 25 years. The most common second cancer reported was a second primary breast cancer, followed by colon cancer, uterine cancer and ovarian cancer (Figure 1.1). Overall, the estimated relative risk of ovarian cancer in women with a prior breast cancer diagnosis was 1.27 (observed/expected (O/E), $p<0.05$) [42]. However, other studies have shown that women with breast cancer have an approximate two-fold increased risk of developing ovarian cancer [34,35,39,43]. Further, in women diagnosed with breast cancer at a young age, this risk increases to more than 5-fold if they have a family history of breast cancer and 17-fold if they have a family history of ovarian cancer [34]. Also using SEER data for the same time period, Freedman and colleagues report that the cumulative incidence of developing any second cancer after ovarian cancer was 9.4% within 25 years. Second primary cancers following ovarian cancer included acute leukemia, breast cancer, colon cancer and bladder cancer. Overall, the estimated relative risk of breast cancer in women with a prior ovarian cancer diagnosis was 1.09 (O/E, $p<0.05$) [44]. However, a study by van Niekerk *et al*. in the Netherlands observed a greater than two-fold increased risk of developing breast cancer within the same year of an ovarian cancer diagnosis [41]. Further research is needed to clarify the

Figure 1.1. Cumulative incidence of developing a second cancer among women with a prior breast cancer diagnosis, SEER 1973-2000 [42]



etiology of double primary breast and ovarian cancer (DPBOC) which may include shared risk factors, genetics, and epigenetics.

1.6. Shared Risk Factors for Breast and Ovarian Cancers

A number of established and suspected risk factors are similar for both breast and ovarian cancer and include advanced age, reproductive factors, family history, and height (reviewed in [45]); the majority are non-modifiable. This dissertation evaluated three potentially modifiable shared risk factors: OC use, parity, and breastfeeding (Tables 1.4 and 1.5). One of the few modifiable preventive options for ovarian cancer is oral contraceptive (OC) use; a 50% lower ovarian cancer risk has been observed in women who use OCs for 10 years or more [46]. Further, this protective effect of OC use has been observed in high risk women with a *BRCA*1/2 mutation [47]. However, OC use has been suspected to increase breast cancer risk [48], although results have been inconsistent [47]. Given these potential discordant effects of OC use on breast and ovarian cancer, research is needed to support clinical recommendations for women from cancer families who are at an increased risk of developing both cancers.

Table 1.4. Reproductive and Hormonal Risk Factors for Breast and Ovarian Cancer in the General Population

Risk Factor	Breast	Ovarian	Comments/Reference
Parity	↓↑	↓	A lower risk of breast cancer after the age of 40 years has been observed for parous women with increasing protection for additional full-term pregnancies; however, a greater risk of very early-onset breast cancer has been observed for parous women compared to nulliparous (reviewed in [45,49,50]). A decreased risk of ovarian cancer has been observed for parous women compared to nulliparous and with each additional full-term pregnancy (reviewed in [45,46]).
Later age at first birth	↑	↓↑⌀	A greater risk of breast cancer has been observed for later age at first parity and may be stronger for pre-menopausal breast cancer compared to post-menopausal [45,49]. The association between age at first parity and ovarian cancer is less clear with studies showing positive, inverse, and null associations [45,46].
Later age at last birth	↑⌀	↓	Most studies have reported a positive association between later age at last parity and breast cancer, while one found no association [49]. An inverse association has been observed between later age at last parity and ovarian cancer [51-54].
Breastfeeding	↓	↓⌀	An inverse association between breastfeeding and risk of breast cancer has been observed with a decreasing trend for increasing duration (reviewed in [45,49]). A lower risk of ovarian cancer has been observed for women who ever breastfed vs. never breastfed, although some literature shows no association

			(reviewed in [45,46]).
Earlier age at menarche	↑	↑ ø	Early age at menarche is associated with a greater risk of breast cancer [45,49]. While many studies have observed a modest increased risk of ovarian cancer with an earlier age at menarche, several studies have also reported a null association [45,46].
Later age at menopause	↑	↑ ø	Later age at menopause is associated with a greater risk of breast cancer [45,49]. While many studies have observed a greater risk of ovarian cancer with later age at menopause, several studies have also reported a null association [45,46].
OC use	↑	↓	OC use has been associated with a greater risk of breast cancer; however this association is more prominent in younger women (25-34 years) and lasts for 10 years after stopping use. A lower risk of ovarian cancer has been observed with OC use and this protection lasts up to 20 years after stopping use (reviewed in [45]).
HRT use	↑	↑ ø	There is a positive association between HRT use and breast cancer [45]. While many studies have observed a greater risk of ovarian cancer with HRT use, several studies have also reported a null association [45,46].

Table 1.5. Reproductive and Hormonal Risk Factors for Breast and Ovarian Cancer in *BRCA*1/2 Mutation Carriers

Risk Factor	Breast	Ovarian	Comments/Reference
Parity	↓↑ ø	↓↑ ø	There have been mixed results for the effect of parity on breast and ovarian cancer in high risk women. For breast cancer, some studies have observed no association in *BRCA*1 carriers and a positive association in *BRCA*2 carriers (reviewed in [50]). For ovarian cancer, some studies have reported no association with ovarian cancer [55], while others have reported either positive associations [56-58] or inverse associations [57,59,60].
Later age at first birth	↑ø	↓	Studies have reported no association [61,62] or a positive association [63,64] between later age at first parity and breast cancer; whereas an inverse association has been reported with ovarian cancer [58,62]. Type of mutation (*BRCA*1 vs. *BRCA*2) may modify the results.
Later age at last birth	-	-	No studies were found assessing age at last birth and breast and ovarian cancer in *BRCA*1/2 carriers.
Breastfeeding	↓ ø	↓ ø	A lower risk of breast cancer has been observed with breastfeeding for *BRCA*1 carriers, but not *BRCA*2 carriers (reviewed in [50]). Similarly, a lower risk of ovarian cancer has been observed for *BRCA*1 carriers, but not *BRCA*2 carriers [57]; however others have observed no association with *BRCA*1/2 carriers [56].
Earlier age at menarche	↑ø	↑	Earlier age at menarche has been shown to have a positive association (reviewed in [50]) [55] and null association with

			breast cancer [65]. There has been a suggestion of a greater risk of ovarian cancer with an earlier age at menarche [55].
Later age at menopause	ø	↑	No association has been observed between age at menopause and breast cancer in *BRCA*1/2 carriers [65]. One study observed a positive association between later age at menopause and ovarian cancer in *BRCA*1/2 carriers [66].
OC use	ø ↑	↓	Case-control studies have showed no association between OC use and breast cancer risk in *BRCA*1/2 carriers; however cohort studies have suggested a positive association in *BRCA*1 carriers [47]. A reduced risk of ovarian cancer has been observed in *BRCA*1/2 carriers [47].
HRT use	ø	ø	One study observed no association between HRT use and breast cancer in *BRCA*1 mutation carriers [67]. Similarly, one study observed no association between HRT use and ovarian cancer in *BRCA*1/2 mutation carriers [68].

1.7. Genes and Breast and Ovarian Cancers

Many breast and ovarian cancer susceptibility genes have been identified in the literature. Table 1.6 lists the established breast cancer susceptibility genes with high, moderate, and low-penetrance [69] and Table 1.7 shows groups of genes that have been identified for Type 1 (low-grade serous carcinomas, borderline serous tumors, low-grade endometriod, and mucinous and clear-cell carcinomas) and Type 2 (high-grade serous carcinomas, carcinosarcomas, and undifferentiated cancers) ovarian tumors [15]. Many genes have been found to be involved in both breast and ovarian cancer including *BRCA*1, *BRCA*2, *TP53*, and *PTEN* which may contribute to the development of DPBOC.

Table 1.6. List of breast cancer susceptibility genes

Penetrance of Pathogenic Variants	Genes
High	BRCA1, BRCA2, TP53, PTEN, LKB1, CDH1
Medium	ATM, CHEK2, BRIP1, PALB2
Low	CASP8, FGFR2, MAP3K1, LSP1, TNRC9, H19

Table 1.7. Genes with pathogenic variants associated with subtypes of ovarian cancer

Ovarian Cancer Subtype	Genes
Type 1	KRAS, BRAF, ERBB2, PTEN, PIK3CA, CTNNB1, ARID1A, and PPP2R1A
Type 2	BRCA1, BRCA2, and TP53

The Cancer Genome Atlas (TCGA) was established as a collaboration between the National Cancer Institute and National Human Genome Research Institute to comprehensively identify key molecular changes across 33 types of cancer, including breast and ovarian. In their breast cancer study using 510 breast tumors, TCGA identified 35 significantly mutated genes (Table 1.8). They analyzed normal tissue on a select group of genes and observed deleterious germline variants in the following genes in approximately 10% of the samples: *ATM*, *BRCA*1, *BRCA*2, *BRIP1*, *CHEK2*, *NBN*, *PTEN*, *RAD51C* and *TP53* [70]. In their ovarian cancer study using 316 high-grade serous ovarian cancer samples and matched normal samples, TCGA identified 9 significantly mutated genes from each individual (Table 1.8) [71]. Genes found to be involved in both breast and ovarian cancer, identified in the TCGA, are highlighted in grey.

Table 1.8. Statistically significant mutated genes in breast and ovarian tumor samples, TCGA

Breast Genes	Ovarian Genes
TP53	TP53
PIK3CA	BRCA1
GATA3	CSMD3
MAP3K1	NF1
MLL3	CDK12
CDH1	FAT3
MAP2K4	GABRA6
RUNX1	BRCA2
PTEN	RB1
TBX3	
PIK3R1	
AKT1	
CBFB	
TBL1XR1	
NCOR1	
CTCF	
ZFP36L1	

GPS2	
SF3B1	
CDKN1B	
USH2A	
RPGR	
RB1	
AFF2	
NF1	
PTPN22	
RYR2	
PTPRD	
OR6A2	
HIST1H2BC	
GPR32	
CLEC19A	
CCND3	
SEPT13	
DCAF4L2	

The TCGA also identified molecular commonalities between basil-like breast tumors and high-grade serous ovarian cancers, including *BRCA*1 inactivation, *RB1* loss and cyclin E1 amplification, high expression of AKT3, MYC amplification and high expression, and a high frequency of *TP53* mutations, suggesting that these are shared molecular events in the development of these two cancers [70].

1.8. Breast and Ovarian Cancer Inherited Susceptibility Syndromes

There are a few established inherited susceptibility syndromes associated with an increased risk of breast and/or ovarian cancer. The most common inherited susceptibility syndrome is hereditary breast and ovarian cancer (HBOC). HBOC is caused by a mutation in *BRCA*1 or *BRCA*2 and is characterized by an increased risk of breast cancer, ovarian cancer, prostate cancer, and pancreatic cancer. Having DPBOC in an individual or family is one of the characteristics identified as indicating high risk of this syndrome. Other characteristics include breast cancer diagnosed before the age of 50, ovarian cancer at any age, multiple primary breast cancers, male breast cancer, triple-negative breast cancer, pancreatic cancer with breast or ovarian cancer in the same individual or on the same side of the family, Ashkenazi Jewish heritage, two or more relatives with breast cancer with one diagnosed before the age of 50, three or more family members with breast cancer at any age, and previously-identified *BRCA*1 or *BRCA*2 mutation in a family [72]. In addition to HBOC there are several other inherited susceptibility syndromes associated with breast and/or ovarian cancer (Table 1.9 adapted from [72]).

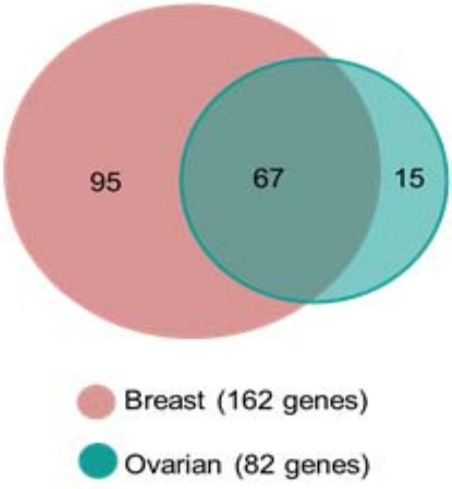
Table 1.9. Inherited susceptibility syndromes of breast and ovarian cancer

Name	Gene	Cancer Association		
		Breast	Ovarian	Other
Li Fraumeni syndrome	*TP53*	Breast	Although ovarian cancer is not often found in families with this syndrome, it has been associated with TP53 mutations	Soft-tissue sarcoma, leukemia, osteosarcoma, melanoma, breast colon, pancreas, adrenal cortex, and brain
Cowden syndrome/*PTEN* Hamartoma tumor syndrome	*PTEN*	Breast		Thyroid, and endometrium
Hereditary diffuse gastric cancer	*CDH1, CHEK2* (1100delC variant)	Breast (male and female)		Prostate, colon, thyroid, and kidney
Ataxia-telangiectasia	*ATM*	Breast		Leukemia, lymphoma,
Lynch syndrome/Hereditary nonpolyposis colorectal cancer	*MLH1, MSH2, MSH6, PMS2*	Suggested association with breast	Ovarian	Colon, endometrium, stomach, pancreas, brain, small bowel, and skin
Peutz-Jeghers syndrome	*STK11*	Breast	Ovarian (often non-epithelial)	Colon and rectum, stomach, small intestine, pancreas
Bloom syndrome	*BLM* (RECQL3)	Breast		Skin, head, neck, esophagus, and gastrointestinal tract
Werner syndrome	*WRN* (RECQL2)	Breast		Sarcomas, melanoma, thyroid cancer, and hematologic malignancies
Xeroderma pigmentosum	*XP* genes A through G	Breast		Cutaneous and ocular malignancies, brain, uterus, testes, and gastrointestinal

1.9. DNA Methylation and Breast and Ovarian Cancer

Alterations in DNA methylation patterns, one common type of epigenetic event, can activate or silence key genes involved in carcinogenesis. Specifically, hypermethylation can result in the silencing of tumor suppressor genes while hypomethylation can activate oncogenes [73-75]. Aberrant DNA methylation is thought to be one of the earliest markers of carcinogenesis [74]. Numerous genes involved in DNA repair, cell cycle regulation and apoptosis have been found to be hypermethylated in breast and ovarian cancer. Further, breast and ovarian cancer share a large number of hypermethylated genes which may contribute to the development of DPBOC. In a review, Heichman *et al*. reported the most common genes hypermethylated in six solid cancers and displayed the cancer pairs with the most overlap. Breast and ovarian cancer have approximately 38% overlap (Figure 1.2) and some of these genes include *APC, BRCA1, E-cadherin, CDKN2A/P16^{INK4A}, DAPK1, ESR1, H1N1, IGFPB3, MLH1, RARβ and RASSF1* [76]; despite this overlap a large number of genes are unique to both. This overlap has yet to be robustly tested in higher risk women as most of the existing studies focus on sporadic cases of breast and ovarian cancers.

Figure 1.2. Venn diagram showing the overlap in hypermethylated genes between breast and ovarian cancer (adapted from Heichman 2012 [76])



1.10. Specific Aims

In order to further our understanding of risk factors for the development of DPBOC, this dissertation addressed the following three aims:

AIM 1: To carry out a comprehensive review of the literature on double primary breast and ovarian cancer (DPBOC) and identify studies that examine risk factors for developing DPBOC, including epidemiologic, genetic, and epigenetic.

AIM 2: To examine the association between hormonal and reproductive risk factors (OC use, parity, and breastfeeding) and risk of 1) second primary ovarian cancer following a breast cancer diagnosis (BR-OV), 2) second primary breast cancer following an ovarian cancer diagnosis (OV-BR), 3) DPBOC (BR-OV and OV-BR combined), 4) single primary breast cancer (BR), and 5) single primary ovarian cancer (OV) in a retrospective case-control study design.

Hypothesis: Parity and breastfeeding will be inversely associated with DPBOC, regardless of diagnosis order, while the net associations with OC use will depend on the underlying genetic/familial risk of breast or ovarian cancer.

AIM 3: To examine the association between hormonal and reproductive risk factors (OC use, parity, and breastfeeding) and risk of: 1) BR-OV, 2) OV-BR, 3) BR, and 4) OV in a prospective cohort study design.

Hypothesis: Parity and breastfeeding will be inversely associated with DPBOC, regardless of diagnosis order, while the net associations with OC use will depend on the underlying genetic/familial risk of breast or ovarian cancer.

With enhancements in screening technology and treatment for cancer, leading to better cancer survival, risk of secondary cancer has become a greater public health issue. Being able to precisely predict whether a woman with breast cancer will go on to develop ovarian cancer, and whether a woman with ovarian cancer will go on to develop breast cancer, is critical for appropriate clinical management and screening of cancer patients, including prophylactic surgery options. However, because of the limitations of current screening methods (i.e., mammography is limited in its screening ability, particularly for women under the age of 50, and no current effective ovarian cancer screening tool exists), primary prevention is critical. This dissertation examined the association between potentially modifiable reproductive and hormonal risk factors and DPBOC in a family-based cohort of breast and ovarian cancer families enriched for increased familial risk.

Chapter 2. Comprehensive Review of Current Literature on Risk Factors for Double Primary Breast and Ovarian Cancer

2.1 Introduction

With enhancements in screening methods for some cancers leading to earlier detection of cancer and improvements in cancer treatment, the number of people living with a prior cancer diagnosis has increased. In the U.S. there are approximately 15 million people who have been diagnosed with cancer and while the incidence of cancer has been declining, the absolute number of people with cancer has been increasing [77]. With improved prognoses and longer survival for individuals with cancer, it is critical to study the risk of a second primary cancer [78].

Second primary cancers may develop from treatment effects of the first cancer, as well as shared host factors such as age or genetics, shared lifestyle or environmental risk factors such as tobacco or contaminants, and interactions between factors, such as gene-environment interactions (Table 2.1).

Table 2.1. Etiology of second primary cancers (Adapted from [79,80])

Shared Risk factors for first primary and second primary cancers		
Lifestyle	**Environmental**	**Host Factors**
Tobacco	Contaminants	Age and Sex
Alcohol	Occupation	Genetics
Diet	Viruses	Immune Function
Other	Other	Hormonal, Other
Treatment of first primary cancer		

Travis and colleagues categorized second primary cancers into three non-mutually exclusive groups based on etiology: 1) treatment-related cancers, 2) syndronomic cancers, and 3) cancers due to shared etiologic exposures [81]. While radiotherapy and chemotherapy have improved the survival for many cancers, research over the past several decades has supported the association between these treatments and the development of second cancers [82]. Syndronomic cancers (discussed in Chapter 1) are associated with major susceptibility genes and are often identified in families. Although syndronomic cancers only account for a small proportion of all second cancers, individuals with mutations in these major susceptibility genes are at a much higher risk of developing multiple primary tumors [81,83]. Tobacco is one of the established environmental exposures associated with the risk of multiple primary

cancers [81,84]. For cancers that share etiologic exposures, the contribution of genetic factors alone may be less and penetrance may be influenced by gene-environment and gene-gene interactions [81]. As second cancers may develop from any of these pathways, including interactions between these pathways, comprehensive research is needed to identify patterns of multiple cancers and to identify individuals at greatest risk.

In the U.S., breast cancer has the highest cancer incidence in females and ovarian cancer is the most fatal of gynecological cancers [85]. Breast and ovarian cancer share many commonalities including some forms of treatment, genes, and epidemiologic risk factors which could contribute to the development of a second primary breast cancer in women with ovarian cancer and a second primary ovarian cancer in women with breast cancer. As developing a second primary cancer is a leading cause of morbidity and mortality in cancer survivors, identifying potentially-modifiable risk factors is critical to reduce its occurrence. My first dissertation aim is to comprehensively review the literature on DPBOC and identify studies that examine risk factors for developing these two primary cancers, including epidemiologic, genetic, and epigenetic.

2.1. Methods

This comprehensive literature review of DPBOC was divided into three parts: 1) epidemiologic, 2) genetic, and 3) epigenetic risk factors. For epidemiologic risk factors a Pubmed search using the following medical subject headings (MeSH) yielded 4,610 results as of February 2018: ("Neoplasms, Second Primary"[MeSH] OR "Neoplasms, Multiple Primary"[MeSH]) AND ("Breast Neoplasms"[MeSH] OR "Ovarian Neoplasms"[Mesh]) Filters: English, Human. After reviewing the titles for relevance and removing studies that did not assess epidemiologic risk factors, including lifestyle, reproductive, and treatment factors, or both breast and ovarian cancer, 143 abstracts were selected for review, yielding 18 studies assessing epidemiologic risk factors for DPBOC.

For genetic risk factors we used the same search terms used for the epidemiologic risk factors which yielded 4,610 results as of February 2018: ("Neoplasms, Second Primary"[MeSH] OR "Neoplasms, Multiple Primary"[MeSH]) AND ("Breast Neoplasms"[MeSH] OR "Ovarian Neoplasms"[Mesh]) Filters: English, Human. After reviewing the titles for relevance and removing studies

that did not assess genetic factors or both breast and ovarian cancer, 18 abstracts were selected for review, yielding 13 studies assessing genetic risk factors for DPBOC.

For epigenetic risk factors a Pubmed search using the following terms yielded 82 results as of February 2018: ("Neoplasms, Second Primary"[MeSH] OR "Neoplasms, Multiple Primary"[MeSH]) AND "DNA methylation "[MeSH] Filters: English. After reviewing the titles for relevance, no abstracts were identified assessing DNA methylation and DPBOC.

2.2. Results

The results from the comprehensive review on epidemiologic risk factor associations with DPBOC are summarized in Tables 2.2 to 2.5 and discussed below.

2.2.1. Reproductive risk factors: Parity

Two studies suggested a lower risk of second primary cancer for parous versus nulliparous women; however there were no statistically significant findings. Specifically, in a study examining risk of second primary ovarian cancer following breast cancer (BR-OV) versus breast cancer only (BR) there was a non-statistically significant lower risk with four or more children compared to nulliparous women (HR=0.67, 95% CI: 0.22, 2.03) [86] and in a study examining the risk of second primary breast cancer following ovarian cancer (OV-BR) versus ovarian cancer only (OV) there was a non-statistically significant greater risk for nulliparous women compared to parous women (RR=1.41, 95% CI: 0.78, 2.56) [87]. In contrast, a study examining DPBOC versus ovarian cancer only observed no statistically significant difference in parity and number of live births; however they only examined bivariable differences [88].

2.2.2. Reproductive risk factors: Age at First Birth

Two studies suggested a greater risk of second primary cancer for women with an older age at first parity versus women with a younger age at first parity; however there were no statistically significant findings. Specifically, in a study examining risk of BR-OV versus BR there was a non-statistically significant greater risk for women with an age at first birth of 35 years and greater compared to less than 25 years of age (HR=2.32, 95% CI: 0.55, 9.72) [86] and in a study of OV- BR versus OV there was a non-statistically significant greater risk for age at first birth greater than or equal to age 30 compared to less

than 30 years (RR=1.43, 95% CI: 0.52, 3.91) [87]. In contrast, a study examining DPBOC versus OV observed no statistically significant difference in age at first birth between groups [88].

2.2.3. Reproductive risk factors: Breastfeeding

A study examining DPBOC versus OV observed no statistically significant difference in length of breastfeeding between groups [88].

2.2.4. Reproductive risk factors: Age at Menarche

A study examining risk of BR-OV versus BR reported a non-statistically significant lower risk for women with an age at menarche of 12 or 13 versus <12 years, but no association for 14 versus <12 years [86]. A study examining risk of OV-BR versus OV observed a non-statistically significant lower risk for women with an age at menarche less than or equal to 13 years compared to greater than 13 years (RR=0.60, 95%CI: 0.29, 1.28) [87]. Cvelbar *et al*. observed no statistically significant difference in age at menarche between women with DPBOC and women with OV [88].

2.2.5. Reproductive risk factors: Menopausal Status

One study observed no association with menopausal status at breast cancer diagnosis and risk of second primary ovarian cancer [86]. Similarly, a study examining DPBOC versus OV observed no statistically significant difference in menopausal status between groups [88]. In contrast, one study observed an increased risk of second primary ovarian cancer for women with pre-menopausal breast cancer versus post-menopausal breast cancer [89]. No studies were identified that examined menopausal status at ovarian cancer diagnosis and risk of second primary breast cancer.

Table 2.2. Summary of reproductive risk factors for double primary breast and ovarian cancer

Author	Pub Date	Study Design	Data source	Study Details	Summary
Parity					
Trentham-Diaz [86]	2007	Cohort	Washington State	9,356 women with breast cancer followed for second cancer, including ovarian cancer (n=36)	Non-statistically significant inverse association for 4+ births vs. nulliparous (HR=0.67, 95% CI: 0.22, 2.03)
Cvelbar [88]	2005	Case-Control	Slovenia	Women with DPBOC (n=31) vs. OV (n=62)	No statistically significant difference between cases and controls for nulliparous vs. parous and number of births (p=0.51)
Bergfeldt [87]	2001	Case-Control	Regional Swedish Cancer Registry	Women with OV-BR (n=72) vs. OV (n=177)	Non-statistically significant positive association for nulliparous vs. parous (RR=1.41, 95% CI: 0.78, 2.56)
Age at First Parity					
Trentham-Diaz [86]	2007	Cohort	Washington State	9,356 women with breast cancer followed for second cancer, including ovarian cancer (n=36)	Non-statistically significant positive association for ≥35 vs. <25 years with risk of BR-OV (HR=2.32, 95% CI: 0.55, 9.72)
Cvelbar [88]	2005	Case-Control	Slovenia	Women with DPBOC (n=31) vs. OV (n=62)	No statistically significant difference between cases and controls for age at first birth (p=0.38)
Bergfeldt [87]	2001	Case-Control	Regional Swedish Cancer Registry	Women with OV-BR (n=72) vs. OV (n=177)	Non-statistically significant positive association with ≥30 vs. <30 years for risk of OV-BR (RR=1.43, 95% CI: 0.52, 3.91)
Breastfeeding					
Cvelbar [88]	2005	Case-Control	Slovenia	Women with DPBOC (n=31) vs. OV (n=62)	No statistically significant difference between cases and controls for length of breastfeeding (p=0.69)
Age at Menarche					
Trentham-Diaz [86]	2007	Cohort	Washington State	9,356 women with breast cancer followed for second cancer, including ovarian cancer (n=36)	Non-statistically significant inverse associations for 12 vs. <12 years (HR=0.53, 95% CI: 0.18, 1.55) and 13 vs. <12 years (HR=0.50, 95% CI: 0.18, 1.41) but not 14 vs. <12 years (RR=0.60, 95% CI: 0.29, 1.28)

Cvelbar [88]	2005	Case-Control	Slovenia	Women with DPBOC (n=31) vs. OV (n=62)	No statistically significant difference between cases and controls for age at menarche (p=0.51)
Bergfeldt [87]	2001	Case-Control	Regional Swedish Cancer Registry	Women with OV-BR (n=72) vs. OV (n=177)	Non-statistically significant inverse association between ≤13 vs. ≥14 years and risk of OV-BR (RR=0.60, 95%CI: 0.29, 1.28)
Menopausal Status					
Trentham-Diaz [86]	2007	Cohort	Washington State	9,356 women with breast cancer followed for second cancer, including ovarian cancer (n=36)	No association between menopausal status and risk of second primary ovarian cancer
Cvelbar [88]	2005	Case-Control	Slovenia	Women with DPBOC (n=31) vs. OV (n=62)	No statistically significant difference between cases and controls for age at menopause (p=0.96)
Langballe [89]	2011	Cohort	Denmark	14,151 women with breast cancer followed for second primary non-breast cancer, including ovarian cancer (n=38)	Increased risk of second primary ovarian cancer in women diagnosed with pre-menopausal (SIR=1.8, 95% CI: 1.2, 2.4) but not post-menopausal breast cancer (SIR=0.8, 95% CI: 0.6, 1.1)

2.2.6. Lifestyle risk factors: OC Use

In a study examining risk of BR-OV versus BR there was no association with OC use (HR= 0.76, 95% CI: 0.34, 1.65) [86].

2.2.7. Lifestyle risk factors: HRT Use

In a study examining risk of BR-OV versus BR there was no association with recent HRT use (HR= 1.36 95% CI: 0.49, 3.73) [86]. Similarly, another study observed no statistically significant difference in HRT use between women with DPBOC and women with only ovarian cancer [88].

2.2.8. Lifestyle risk factors: Smoking

A study examining risk of BR-OV versus BR found current smoking to be associated with a non-statistically significant lower risk (HR= 0.33, 95% CI: 0.10, 1.13) [86].

2.2.9. Lifestyle risk factors: Alcohol Use

In a study examining risk of BR-OV versus BR, any alcohol intake was associated with a statistically significant lower risk (HR=0.45, 95% CI: 0.21, 0.98) [86].

Table 2.3. Summary of lifestyle risk factors for double primary breast and ovarian cancer

Author	Pub Date	Study Design	Data source	Study Details	Summary
OC use					
Trentham-Diaz [86]	2007	Cohort	Washington State	9,356 women with breast cancer followed for second cancer, including ovarian cancer (n=36)	No association with ever OC use for risk of BR-OV (HR= 0.76, 95% CI: 0.34, 1.65)
HRT Use					
Trentham-Diaz [86]	2007	Cohort	Washington State	9,356 women with breast cancer followed for second cancer, including ovarian cancer (n=36)	No association for recent and ever use for risk of BR-OV (HR= 1.36 95% CI: 0.49, 3.73)
Smoking					
Trentham-Diaz [86]	2007	Cohort	Washington State	9,356 women with breast cancer followed for second cancer, including ovarian cancer (n=36)	Non-statistically significant inverse association with current smoking for risk of BR-OV (HR= 0.33, 95% CI: 0.10, 1.13)
Alcohol					
Trentham-Diaz [86]	2007	Cohort	Washington State	9,356 women with breast cancer followed for second cancer, including ovarian cancer (n=36)	Statistically significant inverse association with current alcohol use for risk of BR-OV (HR=0.45, 95% CI: 0.21, 0.98)

2.2.10. Body size risk factors: Weight

In a study examining risk of BR-OV versus BR, having a larger BMI was not associated with risk (HR= 0.80, 95% CI: 0.28, 2.29), for a BMI of greater than or equal to 28.9 kg/m^2 compared to less than 22.5 kg/m^2. Similarly, more adult weight gain was not associated with risk (HR= 1.35, 95% CI: 0.31, 5.84), for gaining more than 36.29kg compared to 0-9.06kg [86].

2.2.11. Body size risk factors: Height

A study examining risk of BR-OV versus BR found no association with height greater than or equal to 1.68m compared to less than 1.60m (HR= 1.30, 95% CI, 0.46, 3.69) [86].

Table 2.4. Summary of body size risk factors for double primary breast and ovarian cancer

Author	Pub Date	Study Design	Data source	Study Details	Summary
Weight					
Trentham-Diaz [86]	2007	Cohort	Washington State	9,356 women with breast cancer followed for second cancer, including ovarian cancer (n=36)	No association with overweight for risk of BR-OV (HR= 0.80, 95% CI: 0.28, 2.29)
Trentham-Diaz [86]	2007	Cohort	Washington State	9,356 women with breast cancer followed for second cancer, including ovarian cancer (n=36)	No association with adult weight loss (HR=1.49, 95% CI: 0.26, 8.47) and high adult weight gain (HR= 1.35, 95% CI: 0.31, 5.84) for risk of BR-OV
Height					
Trentham-Diaz [86]	2007	Cohort	Washington State	9,356 women with breast cancer followed for second cancer, including ovarian cancer (n=36)	No association with greater adult height for risk of BR-OV (HR= 1.30, 95% CI: 0.46, 3.69)

2.2.12. Treatment risk factors: Chemotherapy

Most of the studies identified examined the risk of ovarian cancer following chemotherapy treatment for breast cancer. Only one study observed a non-statistically significant lower risk of ovarian cancer following stage 1 or 2 breast cancer with chemotherapy treatment (RR=0.59, 95% CI: 0.29, 1.21) [90]. The remaining studies observed either no association [91,92] or a suggestive non-statistically significant increased risk of ovarian cancer following chemotherapy treatment for breast cancer [43,93]. However, when chemotherapy was given in combination with radiotherapy, one study showed a statistically significant higher risk of ovarian cancer following breast cancer treatment [43]. Only one study examined risk of breast cancer following chemotherapy treatment for ovarian cancer and they observed no association (RR=0.76, 95% CI: 0.39, 1.46) [87].

2.2.13. Treatment risk factors: Radiotherapy

Most of the studies identified examined the risk of ovarian cancer following radiotherapy treatment for breast cancer. Four studies observed no association between radiotherapy treatment for breast cancer and risk of second primary ovarian cancer [37,92,94,95], two observed non-statistically significant increased risks [93,96], and two observed statistically significant increased risks [43,97]. Two studies examined the risk of breast cancer following radiotherapy treatment for ovarian cancer and both observed no association [87,98].

2.2.14. Treatment risk factors: Tamoxifen

One study observed a non-statistically significant increased risk of ovarian cancer following Tamoxifen treatment for breast cancer (OR=1.79, 95% CI: 0.79, 4.06) [90], and one observed a non-statistically significant increased risk of second primary ovarian cancer following Tamoxifen + radiotherapy treatment for breast cancer (SIR=2.8, 95% CI: 0.2, 149.0) compared to radiotherapy only or surgery only groups (SIR=0.8, 95% CI: 0.1, 3.2) [94]. In contrast, three studies observed no association [92,99,100].

2.2.15. Treatment risk factors: Hormone Therapy

Two studies observed an increased risk of second primary ovarian cancer following hormone therapy for breast cancer, one was statistically significant [101] and one was not [93]; however the two

studies differed because one of them (Yadav and colleagues [93]) examined second primary ovarian and endometrial cancer combined.

Table 2.5. Summary of treatment association with double primary breast and ovarian cancer

Author	Pub Date	Study Design	Data source	Study Details	Summary
Chemotherapy					
Metcalfe [90]	2005	Cohort	10 North American Cancer Genetic Clinics	449 women with stage 1/2 breast cancer with BRCA1/2 followed for ovarian cancer (n=40)	Non-statistically significant inverse association with chemotherapy for risk of BR-OV (OR=0.59, 95% CI: 0.29, 1.21)
Bergfeldt [87]	2001	Case-Control	Regional Swedish Cancer Registry	Women with OV-BR (n=72) vs. OV (n=177)	No association with chemotherapy treatment of ovarian cancer for second primary breast cancer (HR= 0.76, 95% CI: 0.39, 1.46)
Tanaka [93]	2001	Cohort	Osaka Medical Center	2,786 women with breast cancer followed for second primary cancers, including ovarian cancer (n=8)	Non-statistically significant increased risk of second primary ovarian cancer with chemotherapy for breast cancer (SIR=2.8, 95% CI: 0.9, 6.6)
Andersson [92]	2008	Cohort	Denmark	31,818 women with early breast cancer followed for second primary cancers, including ovarian cancer (n=181)	No association between chemotherapy for breast cancer and second primary ovarian cancer in multivariable model (RR=1.28, 95% CI: 0.79, 2.07)
Iwasa [91]	2006	Cohort	Japan	47,005 women with breast cancer followed for second primary cancers, including ovarian cancer (n=27)	No association with chemotherapy for breast cancer and second primary ovarian cancer (p>0.05)
Kirova [43]	2008	Cohort	Institut Curie, France	16,705 women with breast cancer followed for second primary cancers, including ovarian cancer (n=74)	No association with chemotherapy treatment alone for breast cancer and risk of second primary ovarian cancer; increased risk for chemotherapy + radiotherapy (SIR=3.06, 95% CI: 2.07, 4.53)
Radiotherapy					
Bergfeldt [87]	2001	Case-Control	Regional Swedish Cancer Registry	Women with OV-BR (n=72) vs. OV (n=177)	No association with radiotherapy treatment of ovarian cancer and second primary breast cancer (HR=0.70, 95% CI: 0.39, 1.25)
Reimer [98]	1978	Cohort	SEER	18,764 women with ovarian cancer followed for second primary neoplasms, including breast cancer (n=121)	No association with risk of breast cancer following ovarian cancer in irradiated (SIR=1.1, 95% CI: 0.8, 1.5) or non-irradiated groups (SIR=1.0, 95% CI: 0.8-1.4)

Berrington de Gonzalez [97]	2010	Cohort	SEER	182,057 women with breast cancer followed for second primary solid cancers, including ovarian cancer (n=219)	Increased risk of second primary ovarian cancer following breast cancer in Surgery only (SIR=1.27) and Surgery + radiotherapy (SIR=1.43) groups, p<0.05 for both
Tanaka [93]	2001	Cohort	Osaka Medical Center	2,786 women with breast cancer followed for second primary cancers, including ovarian cancer (n=8)	Non-statistically significant increased risk of ovarian cancer following breast cancer with radiotherapy (SIR=3.0, 95% CI: 0.1, 16.7)
Rubino [96]	2000	Cohort	Institut Gustave Roussy, France	4,416 women with breast cancer followed for second primary cancer	Non-statistically significant increased risk of second primary ovarian cancer with radiotherapy for breast cancer (SIR=1.8, 95% CI: 0.9, 3.2)
Andersson [92]	2008	Cohort	Denmark	31,818 women with early breast cancer followed for second primary cancers, including ovarian cancer (n=181)	No association between radiotherapy treatment for breast cancer and second primary ovarian cancer in the multivariable model (RR=1.31, 95% 0.85, 2.01)
Harvey [37]	1985	Cohort	Connecticut	41,109 women with breast cancer followed for second primary cancers, including ovarian and fallopian tube cancers (n=183)	No statistically significant difference in risk of second primary ovarian and fallopian tube cancers between irradiated and non-irradiated breast cancer patients overall, but risk was greater in the radiotherapy group for second cancers diagnosed within one year of the breast cancer (SIR=3.6) compared to the non-radiotherapy group (SIR=1.2)
Grantzau [95]	2013	Cohort	Denmark	46,176 women with early breast cancer followed for second primary cancers, including ovarian, fallopian tube and broad ligament cancers (n=204)	No association between radiotherapy for early breast cancer and second primary ovarian, fallopian tube and broad ligament cancers in multivariable model (HR=1.11, 95% CI: 0.82, 1.51)
Andersson [94]	1991	RCT	Denmark	3,538 women with breast cancer receiving surgical treatment. Women at low risk of recurrence received no further treatment while women at high risk were randomly assigned to either radiotherapy or radiotherapy + tamoxifen and followed for new primary cancers, including ovarian cancer (n=3)	No association with radiotherapy for early breast cancer and second primary ovarian cancer (SIR=0.3, 95%CI: 0.0, 1.9)
Kirova [43]	2008	Cohort	Institut Curie, France	16,705 women with breast cancer followed for second primary cancers, including ovarian cancer (n=74)	Increased risk of ovarian cancer following radiotherapy alone (SIR=1.46, 95% CI: 1.07, 1.98) and radiotherapy + chemotherapy (SIR=3.06, 95% CI: 2.07; 4.53) for breast cancer

Tamoxifen					
Metcalfe [90]	2005	Cohort	10 North American Cancer Genetic Clinics	449 women with stage 1/2 breast cancer with BRCA1/2 followed for ovarian cancer (n=40)	Non-statistically significant positive association with Tamoxifen for ovarian cancer following breast cancer (OR=1.79, 95% CI: 0.79, 4.06)
Cook [99]	1995	Nested case-control	Washington State	Women with breast cancer who develop ovarian (n=39), endometrial (n=42) or contralateral breast cancer (n=234) vs. Random sample of women from the cohort who did not develop a second primary cancer (n=146 controls for ovarian comparison)	No association with Tamoxifen therapy for breast cancer and risk of second primary ovarian cancer (OR=0.6, 95% CI: 0.2, 1.8)
Andersson [92]	2008	Cohort	Denmark	31,818 women with early breast cancer followed for second primary cancers, including ovarian cancer (n=181)	No association between Tamoxifen treatment for breast cancer and second primary ovarian cancer in multivariable model (RR=0.78, 95% CI: 0.41, 1.47)
Curtis [100]	1996	SEER	Cohort	87,323 women with early breast cancer followed for second primary cancers, including ovarian cancer (n=223)	No association between Tamoxifen therapy for breast cancer and second primary ovarian cancer (SIR=0.97, 95% CI: 0.59, 1.50)
Andersson [94]	1991	RCT	Denmark	3,538 women with breast cancer receiving 1) surgery only or randomly assigned to 2) radiotherapy or 3) radiotherapy + tamoxifen and followed for new primary cancers, including ovarian cancer (n=3)	Non-statistically significant increased risk of second primary ovarian cancer following Tamoxifen + radiotherapy treatment for breast cancer (SIR=2.8, 95% CI: 0.2, 149.0) compared to radiotherapy only or surgery only groups (SIR=0.8, 95% CI: 0.1, 3.2)
Hormone Therapy					
Yadav [101]	2009	Cohort	Inst. of Medical Education & Research, India	1,084 women with breast cancer followed for non-breast second cancers, including ovarian cancer (n=7)	Statistically significant positive association with hormone therapy for breast cancer and risk of second primary ovarian cancer (RR=1.14, 95% CI: 1.03, 1.26)
Tanaka [93]	2001	Cohort	Osaka Medical Center	2,786 women with breast cancer followed for second primary cancers, including ovarian cancer (n=8)	Non-statistically significant increased risk of ovarian cancer following breast cancer treatment with hormone therapy (RR=4.85, 95% CI: 0.86, 27.27)

The results from the comprehensive review on genetic associations with DPBOC are summarized in Table 2.6 and discussed below. The majority of studies examining the association between genetic mutations and DPBOC have focused on *BRCA*1/2 mutations and family history.

2.2.16. Genetic risk factors: BRCA1/2

The studies evaluating *BRCA*1/2 mutations in women with DPBOC are diverse making it challenging to compare results across studies. Some of the studies have shown the proportion of women with DPBOC with a *BRCA*1/2 mutation to range from 19% to 85% [102-108]; however, one study did not find a statistically significant greater proportion of *BRCA*1/2 carriers in the case group (DPBOC) than in the control group (women with single primary breast or ovarian cancer) [106]. In a study by Domchek *et al*. examining women with ovarian, peritoneal, or fallopian tube cancer who had *BRCA*1/2 mutations, breast cancer free survival was 97% at 5 years and 91% at 10 years [109]. A small study by Gangi and colleagues observed 8.9% of ovarian cancer cases with a *BRCA*1 or *BRCA*2 mutation developed subsequent breast cancer [110]. A study by Metcalfe *et al*. showed that *BRCA*1 may contribute to the development of DPBOC more than *BRCA*2. Specifically, they observed that women with a *BRCA*2 mutation had a lower risk of ovarian cancer following stage 1 or 2 breast cancer than did those with a *BRCA*1 mutation (RR=0.41, 95% CI (0.19, 0.90)) [90]. In contrast to the studies showing the contribution of *BRCA*1/2 to second primary cancers, Vencken and colleagues observed that the 2-year, 5-year, and 10-year risk of breast cancer was greater among *BRCA*1/2 mutation carriers with no prior cancer (BR) (6%, 16%, and 28%, respectively) than among those with prior ovarian cancer (OV-BR) (3%, 6%, and 11%, respectively) (p=0.03) [111].

2.2.17. Genetic risk factors: Other Genes

In addition to *BRCA*1/2, a few other genes have been examined with DPBOC. Bruchim and colleagues examined p53 expression as a marker for p53 mutations in cases with DPBOC compared to OV and BR controls. They observed no statistically significant differences in p53 expression between the ovarian tissue of cases (68%) and controls (71.9%) or the breast tissue of cases (19.4%) and controls (21.3%) [106]. Pilarski *et al*. observed a higher proportion of the *KRAS*-variant in women with DPBOC who are non-*BRCA*1/2 mutation carriers (27.2%) compared to women with DPBOC who are *BRCA*1 (16.0%) and *BRCA*2 (18.2%) mutation carriers (p<0.001) [107].

2.2.18. Genetic risk factors: Family History

In addition to looking at specific genes, a few studies examined the association between family history and DPBOC. Bergfeldt *et al*. observed a non-statistically significant increased risk of OV-BR for women with a family history of breast and/or ovarian cancer (RR=1.50, 95% CI (0.52, 4.28)) and a statistically significant increased risk with a family history of any cancer (RR=1.94, 95% CI (1.01, 3.72)) [87]. Trentham-Dietz and colleagues observed an increased risk of BR-OV with two or more relatives with breast cancer (HR= 4.28, 95%CI (1.25, 14.6)) [86]. Lastly, two Swedish studies examined the risk of second primary ovarian cancer in women with breast cancer and found contradicting results. Prochazka *et al*. reported an increased risk of BR-OV for women with a family history of breast cancer (SIR=2.32, 95% CI: 1.66, 3.10) compared to women with no family history (SIR=1.38, 95% CI: 1.25, 1.51) [112]. While Hemminki *et al*. also reported an increased risk of BR-OV for women with a family history of breast cancer (SIR=2.0, 95% CI: 1.20, 3.22), it was not statistically significantly different from women with no family history (SIR=1.7, 95% CI: 1.47, 2.03), (p=0.27) [113]. One difference in the studies was their definition of family history. Prochazka *et al*. defined family history as breast cancer in any first degree relative (parent, sibling, offspring) while Hemminki *et al*. defined it as breast cancer in a parent.

Table 2.6. Summary of genetic associations with double primary breast and ovarian cancer

Author	Pub Date	Study Design	Data Source	Outcome	Summary
***BRCA*1/2**					
Gangi [110]	2014	Cohort	Cedars-Sinai Medical Center, Los Angeles	Women with ovarian cancer (n=435) followed for breast cancer (n=12)	8.9% of patients with ovarian cancer with a *BRCA* mutation developed breast cancer after receiving an ovarian cancer diagnosis
Domchek [109]	2013	Cohort	Memorial Sloan-Kettering Cancer Center, University of Pennsylvania	Breast Cancer Free Survival (BCFS) in women with ovarian, peritoneal, or fallopian tube cancer with a BRCA1/2 mutation (n=164)	BCFS was 97% (95% CI: 0.92, 0.99) at 5 years and 91% (95% CI: 0.82, 0.95) at 10 years. In a pseudo-incident cohort where the *BRCA*1/2 test was before or within 1 year after diagnosis (n=64), BCFS was 100% at 5 years and 87% (95% CI: 0.56, 0.96) at 10 years.
Vencken [111]	2013	Cohort	Rotterdam Family Cancer Clinic	Women with ovarian cancer (OV) and a *BRCA*1/2 mutation (n=79) followed for second primary breast cancer (OV-BR) compared to unaffected *BRCA*1/2 mutation carriers (n=351)	The 2-year, 5-year, and 10-year risk of breast cancer was greater for cancer-free women with a *BRCA*1/2 mutation compared to women with ovarian cancer and a *BRCA*1/2 mutation (p=0.03)
Metcalfe [90]	2005	Cohort	10 North American Cancer Genetic Clinics	449 women with stage 1/2 breast cancer with BRCA1/2 followed for ovarian cancer (n=40)	Statistically significant inverse association with *BRCA*2 vs. *BRCA*1 for second primary ovarian cancer following breast cancer (OR=0.41, 95% CI: 0.19, 0.90)
Cvelbar [88]	2011	Cross-sectional	Slovenia	Women with DPBOC (n=167) who agreed to have BRCA1/2 testing (n=20)	60% of women with DPBOC had a *BRCA*1/2 mutation; however only 12% of the cases had genetic testing.
Evans [108]	2010	Cross-sectional	Genetic clinics in North-West England	100 women with DPBOC screened for BRCA1/2 mutations	49% of women with DPBOC tested positive for a *BRCA*1/2 mutation; however only about 20% of women with DPBOC and no family history had a mutation.
Fishman [102]	2000	Cross-sectional	Jewish Israel Women	Women with ovarian cancer with a prior breast cancer (BR-OV) (n=59)	The prevalence of BRCA1/2 mutations was 57% in women with BR-OV
***p*53**					

Bruchim [106]	2004	Cross-sectional	Chaim Sheba Medical Center, Israel	Women with ovarian cancer following breast cancer (BR-OV with an ovarian cancer paraffin block (n=43) vs. women with only ovarian cancer (OV) (n=64)	The proportion of p53 expression was similar between BR-OV cases (69.8%) and OV controls (71.9%).
KRAS					
Pilarski [107]	2012	Cross-sectional	Eight institutions across the US and Ireland	Women with breast cancer and ovarian or fallopian tube or primary peritoneal cancers (DPBOC) with a *BRCA*1 mutation (n=75), *BRCA*2 mutation (n=33), and no *BRCA*1/2 mutation (n=124)	*KRAS*-variant was found in 21% of the entire DPBOC cohort. Prevalence of the *KRAS*-variant was significantly higher in women with DPBOC who were non-*BRCA*1/2 mutation carriers (27.2%) compared to women with DPBOC who were *BRCA*1 carriers (16.0%) and *BRCA*2 carriers (18.2%)
Family History					
Trentham-Diaz [86]	2007	Cohort	Washington State	9,356 women with breast cancer followed for second cancer, including ovarian cancer (n=36)	Statistically significant increased risk of BR-OV with ≥2 relatives with breast cancer (HR=4.28, 95% CI: 1.25, 14.6)
Bergfeldt [87]	2001	Case-Control	Regional Swedish Cancer Registry	Women with OV-BR (n=72) vs. OV (n=177)	Non-statistically significant increased risk of OV-BR with family history of breast or ovarian cancer (RR=1.50, 95% CI: 0.52, 4.28) and statistically significant increased risk of OV-BR with family history of any cancer (RR=1.94, 95% CI: 1.01, 3.72)
Prochazka [112]	2006	Cohort	Sweden	152,600 women with breast cancer followed for second primary cancer, including ovarian cancer (n=712)	Statistically significant increased risk of BR-OV with family history of breast cancer (SIR=2.32, 95% CI: 1.66, 3.10) compared to no family history (SIR=1.38, 95% CI: 1.25, 1.51)
Hemminki [113]	2008	Cohort	Sweden	43,398 women with breast cancer followed for second primary cancers, including ovarian cancer (n=174)	No statistically significant difference in risk of BR-OV for women with a family history (SIR=2.0, 95% CI: 1.20, 3.22) compared to women with no family history (SIR=1.7, 95% CI: 1.47, 2.03)
Fishman [102]	2000	Cross-sectional	Jewish Israel Women	Women with ovarian cancer with a prior breast cancer (BR-OV) (n=59)	The prevalence of having a family history of breast or ovarian cancer in a 1st degree relative was 25.5% in the BR-OV group compared to 10.5% in the OV group (p=0.003)

2.2.19. Epigenetic risk factors

No studies were identified examining DNA methylation and DPBOC.

2.3. Discussion

This comprehensive review highlights the overall lack of research examining risk factors for the development of DPBOC. There were few statistically significant findings evaluating epidemiologic risk factors and risk of DPBOC which may be due to a small number of events within the cohort studies, and small overall sample size in the case-control studies. For reproductive and lifestyle risk factors there was a suggestion of a greater risk of DPBOC for nulliparity, older age at first birth, younger age at menarche, and being pre-menopausal at first primary cancer diagnosis. There was a suggestion of a lower risk of DPBOC with cigarette smoking, and alcohol use. However, with the exception of alcohol use, there were no statistically significant associations with these epidemiologic risk factors and risk of DPBOC. Overall, the majority of studies identified in this literature search assessed cancer treatment as a risk factor for second primary cancer and overall the findings were quite mixed for chemotherapy, radiotherapy and Tamoxifen treatment, while two studies suggested a positive association between hormonal therapy for breast cancer and subsequent ovarian cancer. However, most of these large national database studies assessing the association between treatment of breast cancer and risk of second primary cancers lacked risk factor and family history data and only examined unadjusted associations.

Most studies examining genetic associations with DPBOC have focused on the *BRCA*1/2 genes and family history. While most of these studies confirmed an association between mutations in *BRCA*1/2 and risk of DPBOC, the contribution of these genes varied greatly between studies and may differ by type of mutation (*BRCA*1 vs. *BRCA*2). For other genes, one study observed no association between p53 expression and DPBOC, while another study observed enrichment of the *KRAS* oncogene in women with DPBOC, but it was only enriched in non-*BRCA*1/2 mutation carriers. Having a family history of breast and ovarian cancer was associated with an increased risk of DPBOC. No studies were found examining DNA methylation and risk of DPBOC highlighting the need for research into potential epigenetic pathways.

This comprehensive review has revealed several study limitations and gaps in the literature on DPBOC that Aims 2 and 3 will address. First, few studies have evaluted the association between epidemiologic risk factors and DPBOC; even more limited is research evaluating differences in these associations by cancer diagnosis order (BR-OV versus OV-BR). The studies that have been done are limited by a small sample size for women with both primary cancers, resulting in low power to detect statistically significant associations, and have used different reference groups making comparisons across studies challenging. Further, with many breast and ovarian cancer studies showing differences in risk factor associations between average and high risk women [56-58,65,114,115], it is critical to also evaluate these associations with DPBOC across the risk spectrum. Aims 2 and 3 will address these gaps by evaluating hormonal and reproductive risk factors and risk of DPBOC using a larger sample of cases and evaluating differences by cancer diagnosis order and risk profile using a consistent referent group. Aim 2 will utilize a retrospective case-control study design while Aim 3 will utilize a prospective cohort study design. Evalutating this important research question using two different study designs will allow us to overcome some of the limitations specific to any one study design.

Research on the genetics and epigenetics of DPBOC is sparse. The studies examining genetic associations with DPBOC have mostly focused on *BRCA*1/2 mutations and have not assessed genetic differences by cancer diagnosis order. Further, comparison across these studies is challenging due to their diversity in study design and sample selection. Aims 2 and 3 will assess reproductive and hormonal risk factors for DPBOC across the risk spectrum which is important as most women with a family history of breast or ovarian cancer do not carry mutations in *BRCA1/2*. No studies were found evaluating DNA methylation and DPBOC. While this dissertation will not examine DNA methylation and DPBOC, my future research will examine DNA methylation patterns in women who develop first primary ovarian cancer (OV) and women who develop second primary ovarian after breast cancer (BR-OV), and I have already initiated data collection for this.

Chapter 3. Hormonal and Reproductive Risk Factors for Double Primary Breast and Ovarian Cancer in the Breast Cancer Family Registry, A Retrospective Case-Control Study

3.1. Abstract

Background: Few studies have examined hormonal and reproductive risk factors for double primary breast and ovarian cancer (DPBOC). While oral contraceptive (OC) use has been associated with a reduced risk of ovarian cancer, data have been less consistent with breast cancer. Parity has been consistently associated with a reduced risk of breast and ovarian cancer in average risk women; however results have been conflicting in high risk women. Given these potential discordant effects between cancers and between average and high risk women, we conducted a study to examine the influence of these factors on DPBOC in women across the risk spectrum.

Methods: Using the resources of the Breast Cancer Family Registry, we conducted a case-control study to examine OC use, parity, and breastfeeding and risk of breast cancer prior to ovarian cancer (BR-OV) (n=68), ovarian cancer prior to breast cancer (OV-BR) (n=18), breast cancer only (BR) (n=2,136), ovarian cancer only (OV) (n=214), and controls (n=3,573). We estimated odds ratios (OR) and 95% confidence intervals (CIs) using polytomous logistic regression with a clustered bootstrap approach.

Results: We observed similar associations for BR-OV and OV-BR. Combining these groups into one overall double primary breast and ovarian cancer (DPBOC) group we observed an inverse association with ever OC use (OR=0.38, 95% CI: 0.22, 0.60) and later age at first birth (OR=0.28, 95% CI: 0.05, 0.64), but a positive association with 1 child (OR=2.50, 95% CI: 0.58, 7.56) and ≥2 children (OR=5.78, 95% CI: 2.82, 14.58). There was an inverse association between ever breastfed and DPBOC. When we stratified by *BRCA*1/2 mutation status, the inverse association between OC use and all of our case groups only remained in the *BRCA*1/2 mutation negative group, while the positive association with parity remained regardless of *BRCA*1/2 mutation status. We observed similar findings in our BR and OV groups.

Conclusion: We observed inverse associations between both OC use and breastfeeding and risk of DPBOC and a positive association with parity; however survivor bias may have influenced these results. These findings suggest there may be potentially modifiable factors associated with the risk of DPBOC.

3.2. Introduction

Effective screening methods that lead to earlier detection of cancer coupled with enhanced cancer treatments have resulted in improved prognoses and longer survival for some cancers. With the growing prevalence of people living with a prior cancer diagnosis, the incidence of second primary cancers has increased [78,81] requiring research on the causes of and treatment for these cancers. Women with breast cancer have an increased risk of developing ovarian cancer [34-39,78] and women with ovarian cancer have an increased risk of developing breast cancer [40,41]. Specifically, research has shown the risk of ovarian cancer following a breast cancer diagnosis to be up to two-fold; however, in women with a family history of breast or ovarian cancer this risk has been shown to be 5-fold and 17-fold, respectively [34]. In addition, the risk of breast cancer following an ovarian cancer diagnosis has been shown to be up to two-fold [41,44]; however having a first-degree family history of breast or ovarian cancer or any malignant disease does not appear to further increase this risk [87].

The etiology of double primary breast and ovarian cancer (DPBOC), and the development of multiple primary tumors in general, may include shared risk factors, genetics, and epigenetics. The Cancer Genome Atlas has shown commonalities between basal-like breast tumors and high-grade serous ovarian cancer, including *BRCA*1 inactivation, RB1 loss and cyclin E1 amplification, high expression of AKT3, MYC amplification and high expression, and a high frequency of TP53 mutations [70]. Limited studies have evaluated the contribution of shared risk factors to the development of DPBOC. A number of established and suspected risk factors are similar for both breast and ovarian cancer and include advanced age, reproductive and hormonal factors, family history, and genetics [45]; the majority are non-modifiable. One of the few modifiable preventive options for ovarian cancer is oral contraceptive (OC) use; a 50% lower ovarian cancer risk has been observed in women who use OCs for 10 years or more [46]. Further, this protective effect of OC use has been observed in high risk women with a *BRCA*1/2 mutation [47] and in women with a familial risk of breast and ovarian cancer [58]. However, OC use has been suspected to increase breast cancer risk [47,48,116,117], although results have been inconsistent with some studies observing no association [47,118-121]. Given these potential discordant effects of OC use on breast and ovarian cancer, research is needed to support clinical recommendations for women at high risk of developing breast and ovarian cancer. In addition to OC use,

parity and breastfeeding are potentially modifiable factors that have also been shown to be associated with both breast and ovarian cancer. In average risk women, parity and breastfeeding have been inversely associated with both cancers (reviewed in [46]); however findings have been less consistent in high risk women for both breast [50,115,122-124] and ovarian cancer [55-60]. Given these potential differences in risk factor associations between average and high risk women, further research is needed across the risk spectrum to clarify these associations and to provide accurate clinical information to women for prevention.

In contrast to the abundance of research examining associations between risk factors and breast and ovarian cancer, studies examining risk factors for DPBOC are sparse. Further, the few studies that have been done are inconsistent regarding cancer diagnosis order (i.e., breast cancer prior to ovarian cancer or ovarian cancer prior to breast cancer) or the control group (i.e., women with breast cancer only, or ovarian cancer only, or women with no cancer) making direct comparisons challenging. A limited number of studies have evaluated the association between OC use, parity, and breastfeeding and DPBOC and the results have been inconsistent. A study by Trentham-Diaz *et al*. observed no association between ever OC use and the development of ovarian cancer after breast cancer compared to breast cancer only [86]. For parity, Trentham-Diaz *et al*. observed a non-statistically significant inverse association between 4 or more live births and risk of ovarian cancer after breast cancer compared to breast cancer only [86] and Bergfeldt and colleagues observed a non-statistically significant positive association between nulliparity and risk of breast cancer after ovarian cancer compared to ovarian cancer only [87]. These two studies both found a non-statistically significant positive association between later age at first birth and DPBOC [86,87]. In contrast, a study by Cvelbar *et al*. observed no association between parity, age at first birth, or breastfeeding and DPBOC compared to ovarian cancer only and no family history; however they did not conduct multivariable regression and only examined bivariable differences [88].

As research examining risk factors for the development of DPBOC is sparse, and little to no research has compared risk factor associations by cancer diagnosis order or risk profile, we conducted a study to examine the association between modifiable hormonal and reproductive factors and the development of DPBOC in women across the spectrum of risk.

3.3. Methods

We conducted a case-control study using participants from the three clinic-based sites of the Breast Cancer Family Registry (BCFR) (New York, Philadelphia, and Utah). Further details on the methodology of the BCFR are published elsewhere [125-135]. Briefly, the New York site of the BCFR enrolled affected and unaffected probands with a family history of breast and/or ovarian cancer from local hospitals, organizations, and breast cancer support groups. The Philadelphia site of the BCFR enrolled affected probands with a family history of breast and/or ovarian cancer from the Fox Chase Network of community hospitals, and Cooper Hospital/University Medical Center in Camden New Jersey, and unaffected probands with a family history of breast cancer from the Family Risk Assessment programs at these centers. The Utah site of the BCFR recruited families with three or more cases of breast or ovarian cancer from local clinicians, the Family Cancer Assessment Clinic at Huntsman Cancer Institute, and an ongoing research study. Institutional review boards at each BCFR site approved the study protocol and all participants provided written informed consent at enrollment. Eligibility criteria included one or more of the following: having two or more relatives affected with breast or ovarian cancer, being a female diagnosed with breast or ovarian cancer at a young age, being a female diagnosed with breast and ovarian cancer at any age, being a male with breast cancer, or being a *BRCA*1/2 mutation carrier [126,136]. For this analysis, cases were individuals with a personal history of breast and/or ovarian cancer at baseline and controls were family members unaffected at baseline with no personal history of breast or ovarian cancer.

The BCFR administered an epidemiologic questionnaire to participants at baseline which collected information on demographics, environment, and behavior, including race/ethnicity, height, weight, physical activity, and smoking and alcohol consumption; reproductive factors including menstrual and pregnancy history, breastfeeding, and hormone use; cancer history including breast and ovarian cancer; and surgical procedures of the breast and ovaries. We administered proxy questionnaires to relatives of deceased participants (26.0% of cases (n=753) and 4.9% of controls (n=189)) [126].

The BCFR tested probands affected with breast and/or ovarian cancer for *BRCA*1 and *BRCA*2 mutations. For unaffected probands the BCFR tested the youngest breast or ovarian cancer case in the

family with an available blood sample. If a deleterious mutation was found then other family members with an available blood sample were tested for the same mutation. Myriad Genetic Laboratories, Inc., conducted the genetic tests using full sequence analysis and self-identified Ashkenazi Jewish participants were screened for the three founder mutations, 185delAG and 5382insC in *BRCA*1 and 6174delT in *BRCA*2 [126,136].

Cancer confirmation was sought at each BCFR site through review of medical records, pathology reports, or tumor samples by local pathologists. Pathologists at each BCFR site used standard pathology review forms and were found to have good to excellent agreement between sites [126]. The use of proxies for deceased cases and lack of availability of old tumor samples limited the number of cancers that were able to be confirmed. Among non-proxy cases, 60.0% of breast cancer only (BR) cases and 43.9% of ovarian cancer only (OV) cases were confirmed. For women with both cancers, 35.9% of breast prior to ovarian cancer (BR-OV) cases and 9.1% of ovarian prior to breast cancer (OV-BR) cases had both cancers confirmed; 60.4% of BR-OV cases and 36.4% of OV-BR cases had at least one cancer confirmed.

3.3.1. Statistical Analysis

We examined mean and percent differences between our exposures of interest (OC use, parity, and breastfeeding) and potential confounding variables with our outcomes of interest (BR-OV, OV-BR, BR, OV, and controls with neither breast nor ovarian cancer). We examined multivariable associations between our exposures and outcomes of interest using unordered polytomous logistic regression with a clustered bootstrap [137] to adjust our standard errors given the correlated nature of our family-based data. For our main analyses, the same control group was used for each of our case groups. However, as a sensitivity analysis, we ran case-case analyses where we compared our double primary groups (BR-OV and OV-BR) to our single primary groups (OV and BR, respectively). We examined the following variables for possible confounding: age, race/ethnicity, age at menarche, BMI (kg/m^2), height (m), education, cigarette use, alcohol use, menopausal status, and BCFR site. We included age as an *a priori* confounder and evaluated the other variables for confounding based on the 10% change-in-beta criterion. All exposures and potential confounders were truncated to one year prior to diagnosis for cases and to date of interview for controls. For individuals missing *BRCA*1/2 mutation data (n=2,293 (34.1%)), we

attempted to impute mutation data based on BOADICEA *BRCA*1/2 carrier probabilities [138]. We used the BOADICEA software package (https://pluto.srl.cam.ac.uk/cgi-bin/bd3/v3/bd.cgi) to calculate a woman's probability of being a *BRCA*1 or *BRCA*2 carrier. Specifically, if a woman had a *BRCA*1 or *BRCA*2 carrier probability of ≥0.5 then we categorized her as being *BRCA*1/2 mutation positive; otherwise they were categorized as *BRCA*1/2 mutation negative. We chose this cutpoint based on our data comparing known genetic test results to the BOADICEA predicted carrier probabilities. Using a *BRCA*1 or *BRCA*2 carrier probability of 0.5 as the cutoff, the sensitivity and specificity were 70.9% and 99.3%, respectively. In addition, we used this software package to determine a woman's 10-year risk of breast and ovarian cancer starting one day prior to diagnosis for cases and at the age at interview for controls. There was a high correlation between the 1-, 5-, and 10-year risks (>95%) in a cohort including non-proxy participants from this study (personal communication, Dr. Terry); therefore if the 10-year risk was missing we imputed using the next available preceding risk score. As a sensitivity analysis, we stratified our analyses by a 20% risk of breast or ovarian cancer as this defines a woman as being at increased risk according to the National Comprehensive Cancer Network guidelines for breast cancer screening [139]. We stratified our analyses by an age 40 cutpoint (Table 3) to examine differences between early and late onset cancers. Menopausal status was determined based on a pre-defined algorithm involving time of last menstrual period, prior bilateral oophorectomy, hormone replacement therapy use, pregnancy, hysterectomy, and age. For individuals missing menopausal status due to missing variables in the algorithm, we imputed the data based on the 90th percentile of age at natural menopause in the controls for smokers (54 years) and non-smokers (55 years) [140].

Overall, we had 106 DPBOC, 2,504 BR, and 285 OV cases, and 3,825 controls eligible for the study; however cases and controls were excluded if they were missing any of the final covariate data. Therefore, our study included 86 DPBOC (81.1%), 2,136 BR (85.3%), and 214 OV (75.1%) cases, and 3,573 controls (93.4%). We had 29 DPBOC, 636 BR, and 74 OV cases from Philadelphia, 36 DPBOC, 1,202 BR, and 100 OV cases from New York, and 21 DPBOC, 298 BR, and 40 OV cases from Utah eligible for the analysis. We had 11 participants with the same diagnosis date for breast and ovarian cancer and kept these participants as a separate group in our initial analyses. However, the direction and magnitude of the main associations were similar for this group as the BR-OV so we combined these

groups to enhance statistical power. Interaction was assessed on the multiplicative scale using cross-product terms and either the Wald Test or Likelihood Ratio Test and on the additive scale using the relative excess risk due to interaction (RERI) [141].

We performed the following sensitivity analyses to test the robustness of our results: 1) excluded women with any other cancer diagnosis (non-breast and non-ovarian cancer) prior to the breast or ovarian cancer diagnosis for cases and prior to the date of interview for controls (Supplemental Table 3.1); 2) excluded proxy data (Supplemental Table 3.2); 3) excluded imputed menopausal status data (Supplemental Table 3.3); 4) excluded imputed *BRCA*1/2 mutation data (Supplemental Table 3.4); 5) excluded women with a prior bilateral oophorectomy or bilateral mastectomy (Supplemental Table 3.5); 6) excluded unconfirmed cases (Supplemental Table 3.6); 7) excluded non-epithelial ovarian cancers (Supplemental Table 3.7); 8) excluded *in situ* breast cancers (Supplemental Table 3.8); 9) excluded synchronous cancers (breast and ovarian cancers diagnosed within 12 months of each other) (Supplemental Table 3.9). Women who have a bilateral oophorectomy are still at risk for breast cancer and women who have a bilateral mastectomy are still at risk for ovarian cancer. Since minimal differences were observed when we excluded these participants (193 cases (6.7%) and 330 controls (8.6%) with prior bilateral oophorectomy; 18 cases (0.6%) and 18 controls (0.5%) with prior bilateral mastectomy), we chose to include them in the final analyses given our multinomial outcome. Further, we observed minimal differences in our results for each of these sensitivity analyses and therefore kept all participants and imputed data in our main analyses to enhance our statistical power. Statistical significance was determined using a p-value cutpoint of 0.05. All analyses were conducted using SAS version 9.3.

3.4. Results

Table 3.1 reports demographic, reproductive, and behavioral differences between the breast/ovarian case groups and controls. Women with OV-BR tended to be older than controls and BR-OV cases. While the majority of our sample was white, women with BR-OV had the highest percentage of whites compared to non-whites. Compared to controls, all of our case groups were more likely to be post-menopausal, to have never consumed alcohol (except BR-OV), to be parous, and to have never used

oral contraceptives. The median time between cancer diagnoses was 7 and 8 years for women with BR-OV and OV-BR, respectively.

Table 3.2 reports our multivariable results assessing the association between OC use, parity, and breastfeeding and risk of BR-OV, OV-BR, and DPBOC (combined groups). OC use was associated with a statistically significant 62% lower risk of DPBOC overall, a 60% lower risk of BR-OV, and a 70% lower risk of OV-BR. For all of our case groups, parity was positively associated with risk of cancer. Having two or more children was statistically significantly associated with a more than 5-fold higher risk of DPBOC, a more than 6-fold higher risk of BR-OV, and a nearly 4-fold higher risk of OV-BR. Having an older age at first birth (>29 years) was associated with a statistically significantly 72% lower risk of DPBOC overall and a 71% lower risk of BR-OV. We observed no association between age at last birth and any of our case groups. For breastfeeding, we observed a 48% lower risk of developing DPBOC and a 51% lower risk of BR-OV. We further examined the impact of parity and breastfeeding using a combined variable on our outcome groups. While we still observed a positive association between parity and DPBOC, regardless of breastfeeding status, the association was attenuated in parous women who ever breastfed compared to parous women who never breastfed (Supplemental Table 3.10).

Table 3.3 reports our multivariable model stratified by *BRCA*1/2 mutation status and age at diagnosis (cases) or interview (controls). For these analyses we did not assess cancer diagnosis order and only present the DPBOC group to enhance our statistical power. Overall, there were minimal differences in risk between the case groups for parity and breastfeeding. However, the inverse association between OC use and DPBOC was only observed in the *BRCA*1/2 mutation negative group and we observed multiplicative interaction between OC use and *BRCA*1/2 mutation status for our DPBOC group ($p=0.02$). There was no multiplicative or additive interaction between parity or breastfeeding and *BRCA*1/2 mutation status for our DPBOC group. There were minimal differences in the results between the women aged less than or equal to 40 years and the women aged greater than 40 years and no multiplicative or additive interaction was observed for our DPBOC group.

To further explore differences across the risk spectrum, we stratified our results by 10-year risk of breast or ovarian cancer using the BOADICEA risk prediction model (Supplemental Table 3.11). The

positive association between parity and DPBOC was greater in women with a risk of 20% or more (high risk) compared to women with a less than 20% risk (average risk). While we did not observe a statistically significant interaction between parity and risk score on either the additive or multiplicative scale, we would expect interaction on at least one scale as both parity and risk score were associated with DPBOC. Therefore, it is likely we did not have sufficient power to detect this interaction. Further, the inverse association between breastfeeding and DPBOC was attenuated and no longer statistically significant in average risk women and we observed additive interaction (RERI = -5.33 (-10.55, -0.12)). Lastly, the inverse association between OC use and risk of DPBOC was attenuated and no longer statistically significant in the high risk group (multiplicative interaction: p=0.04).

Since our parity results disagreed with our hypothesis of an inverse association between parity and risk of DPBOC, and disagrees with much of the breast and ovarian cancer literature showing an inverse association between parity and risk of BR and OV, we conducted additional post hoc analyses in which we assessed differences by number of first and second degree relatives as a proxy for family size (Supplemental Table 3.12), BCFR site (Supplemental Table 3.13), time since last parity, defined as the time between age at last parity and age at diagnosis for cases and age at interview for controls (Supplemental Table 3.14), time between age at menarche and age at first parity (Supplemental Table 3.15), and birth cohort (Supplemental Table 3.16). The positive association between parous women with two or more children and DPBOC and BR remained regardless of family size and BCFR site; however, we were limited by a small sample size in some of the groups. Compared to women with 20 years or more since last parity, women with 10 to less than 20 years since last parity had an increased risk of DPBOC. We also observed an increased risk of BR for women with 10 to less than 20 years and less than 10 years compared to 20 years or more since last parity. We observed no association between time between menarche and first parity and any of our case groups. Lastly, we evaluated differences by birth year, stratifying by being born before 1930, between 1930 and 1960, and after 1960. We chose these cutpoints to examine differences between women exposed to OC formulations with high levels oestrogen (pre-1975) and low levels of oestrogen (post-1975) and to examine potential differences in reproductive habits. In contrast to our main OC findings, for women born prior to 1930 there was no association between OC use and DPBOC and the inverse association between OC use and BR was attenuated and

no longer statistically significant. In addition, the positive association observed between parity and all of our case groups was attenuated and no longer statistically significant. Due to a small sample size, we were unable to examine the association between parity and DPBOC in women born after 1960.

In order to examine differences between women who developed two primary cancers (BR-OV and OV-BR) and women who developed one primary cancer (BR and OV), and to minimize possible survivor bias, we ran case-case analyses for our main exposures (Supplemental Table 3.17). There was a non-statistically significant inverse association between ever OC use and risk of BR-OV versus BR but no association versus OV. There was a non-statistically significant inverse association between ever OC use and risk of OV-BR versus OV, and OV-BR versus BR. We observed a positive association between having two or more live births and all of the double primary cancer groups compared to the single primary cancer groups, but it was only statistically significant for the BR-OV versus BR and versus OV comparisons. Lastly, we observed a non-statistically significant inverse association between ever breastfed and all of the double primary cancer groups compared to the single primary cancer groups.

3.5. Discussion

In our study of women with a familial risk of breast or ovarian cancer we were able to examine the association between hormonal and reproductive risk factors and risk of DPBOC by cancer diagnosis order and risk profile. We observed a lower risk of developing DPBOC (regardless of cancer diagnosis order) with ever OC use; however, when we stratified by *BRCA*1/2 mutation status we only observed these associations in the *BRCA*1/2 mutation negative group. As most women with a family history of breast or ovarian cancer do not carry mutations in *BRCA*1/2 (approximately 75% for breast [24-26] and 56% for ovarian [27]), we further examined our findings by 10-year risk of breast and ovarian cancer and observed a similar inverse association between ever OC use and DPBOC for women at average risk. While there was still a lower risk of DPBOC with ever OC use in the high risk group, it was not statistically significant; however we were limited by a small sample size. We observed a positive association between having two or more children compared to being nulliparous and risk of BR-OV, OV-BR, and DPBOC overall. Additionally, we found an inverse association between later age at first parity and DPBOC. Lastly, we observed an inverse association between breastfeeding and DPBOC in our main analyses.

Our finding of an inverse association between OC use and BR-OV compared to controls contrasts the finding by Trentham-Diaz and colleagues that showed no association with OC use and risk of BR-OV compared to BR [86]; however our reference groups differed. In order to compare our findings using the same reference group we evaluated the association between OC use and BR-OV compared to BR, a case-case analysis, and observed a suggestion of a lower risk but it was not statistically significant (OR=0.63, 95% CI: 0.36, 1.10) (Supplemental Table 3.17). Similar results to our main findings were also found for the BR and OV groups. OC use has been consistently shown to reduce the risk of ovarian cancer in both the general population [142] and in women at high risk [47,143,144] and our findings in women with OV are consistent with this. In contrast, OC use in the general population has been shown to slightly increase the risk of breast cancer, particularly among current or recent users [145]; however findings in high risk women have been inconsistent [47,144]. We observed a lower risk of BR with OC use; however this association only remained in the *BRCA*1/2 mutation negative and average risk groups. While our finding of a reduced risk of BR with ever OC use is not consistent with most studies, one study found a reduced risk of breast cancers in women with a *BRCA*1/2 mutation [146] and one study found a reduced risk of mucinous breast cancer with ever OC use [4].

Our findings of a positive association between having two or more children compared to being nulliparous and risk of BR-OV and OV-BR contradict findings by Trentham-Diaz *et al*. [86] and Bergfeldt *et al*. [87] who found an inverse association between parity and BR-OV and OV-BR, respectively; however their results were not statistically significant. For a more accurate comparison of their findings, we examined our results using similar reference groups, a case-case analysis, and observed a more than 2-fold increased risk of BR-OV compared to BR (OR=2.68, 95% CI: 1.19, 6.73) and OV-BR compared to OV (OR=2.34, 95% CI: 0.64, >999) with two or more children (Supplemental Table 3.17). We also observed a positive association between parity and BR. Parity has been shown to reduce the risk of breast cancer in the general population after age 40 years [49,115,122]; however, results in high risk women have been less consistent. A lower risk of breast cancer has been observed in *BRCA*1 mutation carriers with 4 or more children [115,122] and parous women with a family history of breast cancer compared to parous women with no family history [147], while in *BRCA*2 mutation carriers being parous has been shown to increase the risk of breast cancer [122]. Further, Work *et al*. examined risk factor

associations in less common histologic subtypes of breast cancer and observed that having a greater number of children was positively associated with medullary breast cancer, which is more commonly found in women with *BRCA*1/2 mutations [4].

Since our parity results disagreed with our hypothesis of an inverse association between parity and DPBOC, we conducted additional post hoc analyses to further explore our findings. We found that women with 10 to less than 20 years since last parity had an increased risk of DPBOC compared to women with 20 years or more since last parity and women with 10 to less than 20 years and less than 10 years since last parity had an increased risk of BR compared to women with 20 year or more since last parity. These results suggest that our parity findings may partly be driven by cancers diagnosed closer to pregnancy, which supports prior literature showing a transient increase in breast cancer risk following pregnancy [148,149]. Lastly, for women born prior to 1930 and exposed to high oestrogen OC formulations, there was no association between OC use and DPBOC and the inverse association between OC use and BR was attenuated and no longer statistically significant. In addition, the positive association observed between parity and BR was attenuated and no longer statistically significant for one child and null for two or more children, suggesting a possible birth cohort effect.

It can be difficult to disentangle the independent effects of OC use and parity as trends have shown decreasing parity with increasing OC use [150]. To address this issue we conducted two additional analyses. Supplemental Table 3.18 shows our results looking at duration of OC use in parous women in relation to their first pregnancy. We observed an inverse association between OC use and all of our case groups for women who used OCs only prior to first pregnancy and women who used OCs after first pregnancy. Supplemental Table 3.19 shows our results looking at parity in women who have used OCs by duration of use. We still observed a positive association between parity and risk of DPBOC and BR regardless of duration of OC use. This suggests that the increased risk observed for parous women is not due to a shorter duration of OC use.

Russo and colleagues have shown that the breast tissue architectural pattern of parous women at high risk of breast cancer (either a *BRCA1* mutation or a family history of breast cancer) was similar to nulliparous women at high risk, and these patterns were different from parous women at average risk of

breast cancer [151]; suggesting that the influence of parity on breast cancer risk may have differential effects based on genetic or familial risk status. However, in the present study, we observed an increased risk of DPBOC and BR regardless of *BRCA*1/2 mutation status and breast and ovarian risk score, although the associations were stronger in the higher risk groups (*BRCA*1/2 mutation positive and risk score ≥20%).

Our finding of an inverse association between later age at first parity and DPBOC also contradicts findings by Trentham-Diaz *et al*. [86] and Bergfeldt *et al*. [87] who observed a positive association between later age at first parity and BR-OV and OV-BR, respectively; however their results were not statistically significant. We again examined our results using matching reference groups and observed similar findings to our main analyses (data not shown). Earlier age at first birth has been associated with a reduced risk of breast cancer in average risk women [152], while studies in high risk women have been mixed. For familial breast cancer, one study observed a reduced risk with an earlier age at first parity [147], while a study in *BRCA*1/2 mutation carriers observed no statistically significant association [62]. The association between age at first parity and ovarian cancer in average risk women is less clear, with studies showing positive [45,46,153-162], inverse [45,46,53,163-165], and null associations [45,166], whereas an inverse association [62] and no association [58] have been reported for ovarian cancer in high risk women.

We observed an inverse association between breastfeeding and DPBOC which contradicts the finding of Cvelbar *et al.* [88] of no association. This inverse association remained in stratified analyses of women with a breast/ovarian cancer risk ≥20%, which is consistent to what others have observed in high risk women (*BRCA*1 mutation carriers) [122-124]. When we stratified by *BRCA*1/2 mutation status, we did observe a stronger inverse association between breastfeeding and DPBOC in the mutation positive group, but it did not reach statistical significance. Further, we observed some differences by study site. Specifically, there was a statistically significant inverse association between breastfeeding and DPBOC in the Philadelphia and Utah sites, but not the New York site, highlighting potential differences in the women recruited between sites.

Although pathology collection was attempted on all cases, complete information was not obtained on all due to the use of proxies for deceased cases and availability of pathology for cases diagnosed many years prior. Without confirmation on all cancers, we were unable to confirm that all self-reported cancers in the DPBOC group were primary tumors rather than metastases. However, research has shown that ovarian metastases to the breast are an uncommon event [167,168] and ovarian cancer in a woman with prior breast cancer is more likely to be a second primary cancer than a breast metastasis [169,170]. Additionally, breast metastases to the ovary occur more commonly with lobular than ductal breast carcinomas which are less common [170]. Further, in order to address this concern we excluded synchronous breast and ovarian cancers diagnosed within 12 months of each other and found minimal differences in our results (Supplemental Table 3.9). We were also unable to examine our breast and ovarian cancers by molecular or histological subtype due to incomplete pathology information on our cases. In order to address some of these concerns we ran our analyses limited to only confirmed cases (Supplemental Table 3.6), to only epithelial ovarian cancers (Supplemental Table 3.7) and excluding *in situ* breast cancers (Supplemental Table 3.8) and observed minimal differences in our results, suggesting that these cases were representative of all cases.

Treatment data were collected via self-report in this study and we had missing information on 20.5% of our BR cases, 56.1% of our OV cases, and 30.2% of our DPBOC cases regarding treatment for the first cancer. As some studies have reported an association between treatment and risk of BR-OV and OV-BR we performed a sensitivity analysis with our available data. Specifically, we stratified by any chemotherapy, radiotherapy, or hormone therapy and surgery only. Overall we observed minimal differences in our results with the exception that there was no association between ever breastfeeding and DPBOC in the surgery only stratum (Supplemental Table 3.20). We only had tumor stage data on a limited number of cases so we were unable to assess this. One study found differences in ovarian tumor stage depending on whether the cancer was a first primary or second primary. Specifically the OV-BR group had a higher proportion of stage I and II ovarian tumors compared to the BR-OV group. In contrast they reported a similar proportion of stage I and II breast tumors in the BR-OV and OV-BR groups [171].

Another limitation of this study was the potential role of selection and information bias. First, our analyses included prevalent cases, which may have led to survivor bias. In order to address this concern we conducted pseudo-incident analyses where we stratified by time between diagnosis and interview and examined only cases diagnosed within 2 years of the baseline interview (Supplemental Table 3.21) and within 5 years of the baseline interview (Supplemental Table 3.22). Most of our DPBOC cases were diagnosed more than 5 years before the baseline interview so we had very little power to examine this outcome in these analyses. With the limited number of pseudo-incident DPBOC cases, this analysis only suggested a possible attenuated association for OC use and parity in the pseudo-incident cancers compared to the prevalent cancers. However, as both the first and second primary cancers were retrospective, stratifying by time between diagnosis and interview for both cancers also stratifies by time between diagnosis of the first primary cancer and diagnosis of the second primary cancer. One study has shown that risk of ovarian cancer following ER negative breast cancer, but not ER positive breast cancer, increases with time since the breast cancer diagnosis [38]. Therefore, by stratifying by time between breast and ovarian cancer diagnosis, this analysis could also be stratifying by breast and ovarian cancers with different molecular characteristics and underlying risk of second primary cancer. To address this concern we limited the analysis to just one cancer diagnosed ≤2 years before baseline versus one cancer diagnosed >2 years before baseline and we similarly observed an attenuated association for OC use and parity in the pseudo-incident group compared to the prevalent group (data not shown). When we examined survivor bias in our BR and OV findings, OC use was not associated with pseudo-incident BR or OV but the inverse association remained in the prevalent BR and OV cases. While the positive association between parity and BR remained, it was attenuated in the pseudo-incident case group. When examining differences between our pseudo-incident and prevalent BR and OV cases we found that the pseudo-incident BR and OV cases were more likely to be ever OC users compared to the prevalent cases. Further, the pseudo-incident BR cases were more likely to be pre-menopausal and the pseudo-incident OV cases were more likely to be *BRCA*1/2 mutation positive compared to the prevalent cases. Lastly, there were a higher proportion of high risk women with pseudo-incident DPBOC and OV compared to prevalent DPBOC and OV which could have contributed to some of these observed differences (Supplemental Figure 3.1). Second, given the recruitment methods of this study, it is possible

that selection bias through the selection of controls may have influenced our results. Specifically, cases were recruited from various hospitals, clinics, and centers and their family members were subsequently enrolled. Therefore, cases and controls were not necessarily recruited from the same source population which could have introduced selection bias into the study. Furthermore, without a defined source population we were unable to perform alternative analytic techniques, such as inverse probability weighting, to evaluate the presence of selection bias. However, we were able to replicate the established protective effect of OC use on ovarian cancer risk which reduces the likelihood of strong selection bias. Also, we observed minimal differences between the Utah site, where families in the study are larger and more likely to live locally, and the New York and Philadelphia sites. One way to avoid these selection bias concerns with a case-control design is to conduct a case-case analysis which removes the potential for bias in the selection of controls (women unaffected with breast or ovarian cancer). In this study we also conducted case-case analyses comparing the double primary cancer groups to the single primary cancer groups, and overall we observed similar results to our main findings using controls with no breast or ovarian cancer with the exception of the finding of no association between ever OC use and risk of BR-OV versus OV. However, while these case-case analyses limited the potential for survivor bias as both the case and referent groups had prevalent breast and/or ovarian cancer, the double primary groups had a longer time between first cancer diagnosis and baseline interview (12 and 18 years for BR-OV and OV-BR, respectively) compared to the single primary groups (2 and 4 years for OV and BR, respectively), suggesting that this analysis was not able to entirely rule out survivor bias.

Information bias may have also influenced our results. Recall bias is a concern in case-control studies as exposure information is collected after the outcome and exposure information may be differentially recalled by cases and controls. Parity and breastfeeding are major life events and less susceptible to recall bias. Furthermore, for breastfeeding we used a crude ever/never categorization which would have minimized any information bias. Taking oral contraceptives, on the other hand, may be more susceptible to recall bias compared to parity and breastfeeding. However, oral contraceptive use was also defined as ever/never which would have limited exposure misclassification. Providing further support of minimal information bias in our study parity [172], breastfeeding [173], and OC use [174,175] have been shown to be reliability reported. The case-case analyses would have also minimized recall

bias as both the case and referent groups were individuals with a cancer diagnosis; however differences in the time between cancer diagnosis and entry into the study between groups suggest that this analysis was not able to entirely rule out recall bias.

Unlike prior studies, our study was uniquely able to examine risk factor associations for DPBOC by cancer diagnosis order and risk profile. We observed similar associations between OC use, parity, and breastfeeding and BR-OV and OV-BR, suggesting that these exposures similarly affect the risk of a second primary breast cancer in women with ovarian cancer as with a second primary ovarian cancer in women with breast cancer. Further, we observed some differences in our risk factor associations between women who developed DPBOC and women who developed BR or OV. Specifically, later age at first birth and breastfeeding showed stronger inverse associations with DPBOC, while parity showed a stronger positive association with DPBOC than BR and OV. Given the conflicting literature on parity and breast and ovarian cancer in high risk women, further work is needed to provide clear clinical guidelines to women at greatest risk of developing these cancers. Our findings suggest there may be modifiable factors that can influence a woman's risk of developing DPBOC.

3.6. Tables

Table 3.1. Demographic, reproductive and behavioral differences between ovarian and/or breast cancer cases and controls in the Breast Cancer Family Registry

Characteristic	BR-OV (n = 68)	OV-BR (n = 18)	BR (n =2,136)	OV (n = 214)	All Controls (n = 3,573)
	Mean (S.D.)/n(%)	Mean (S.D.)/n(%)	Mean (S.D.)/n(%)	Mean (S.D.)/n(%)	Mean (S.D.)/n(%)
Age at dx/interview or death (yr)	44.3 (10.6)	47.6 (11.8)	47.9 (12.1)	51.3 (12.5)	44.8 (15.6)
Missing n (%)	0	0	0	0	0
Race (%)					
White	64 (94.1)	13 (72.2)	1,661 (77.8)	173 (80.8)	2,862 (80.1)
Other	4 (5.9)	5 (27.8)	468 (21.9)	39 (18.2)	698 (19.5)
Missing	0	0	7 (0.3)	2 (0.9)	13 (0.4)
Age at menarche (yr)	12.3 (1.3)	12.4 (0.5)	12.5 (1.5)	12.5 (1.7)	12.7 (1.5)
Missing n (%)	17 (25.0)	8 (44.4)	292 (13.7)	72 (33.6)	159 (4.5)
Menopausal Status					
Pre-menopausal	39 (57.4)	10 (55.6)	1,128 (52.8)	101 (47.2)	2,405 (67.3)
Post-menopausal	29 (42.7)	8 (44.4)	1,008 (47.2)	113 (52.8)	1,168 (32.7)
Missing n (%)	0	0	0	0	0
Height (m)	1.6 (0.1)	1.6 (0.1)	1.6 (0.1)	1.6 (0.1)	1.6 (0.1)
Missing n (%)	0	0	8 (0.4)	1 (0.5)	32 (0.9)
BMI (kg/m2)	26.7 (6.7)	25.9 (5.5)	25.9 (5.5)	25.3 (5.3)	25.2 (5.5)
Missing n (%)	1 (1.5)	0	28 (1.3)	5 (2.3)	56 (1.6)
Cigarette Use (%)					
Ever	28 (41.2)	8 (44.4)	879 (41.2)	68 (31.8)	1,257 (35.2)
Never	40 (58.8)	10 (55.6)	1,250 (58.5)	144 (67.3)	2,302 (64.4)
Missing	0	0	7 (0.3)	2 (0.9)	14 (0.4)
Alcohol Use (%)					
Ever	28 (41.2)	2 (11.11)	841 (39.4)	56 (26.2)	1,462 (40.9)
Never	40 (58.8)	16 (88.9)	1,295 (60.6)	158 (73.8)	2,111 (59.1)
Missing	0	0	0	0	0
Parity (%)					
0	10 (14.7)	3 (16.7)	374 (17.5)	39 (18.2)	1,069 (29.9)
1	6 (8.8)	0 (0)	267 (12.5)	18 (8.4)	411 (11.5)
≥2	52 (76.5)	15 (83.3)	1,495 (70.0)	157 (73.4)	2,093 (58.6)
Missing	0	0	0	0	0
Age at first birth (yrs)	23.2 (3.7)	23.3 (3.2)	24.9 (4.9)	24.3 (4.4)	25.1 (5.1)
Missing n (%)	0	0	6 (0.3)	1 (0.6)	7 (0.3)
Age at last birth (yrs)	29.3 (5.2)	30.9 (4.0)	30.4 (5.1)	31.0 (5.1)	30.4 (5.3)
Missing n (%)	1 (1.7)	0	15 (0.9)	1 (0.6)	10 (0.4)
Breastfeeding (BF) (%)					
Ever BF	33 (48.5)	9 (50.0)	1,010 (47.3)	115 (53.7)	1,804 (50.5)
Never BF	35 (51.5)	9 (50.0)	1,126 (52.7)	99 (46.3)	1,769 (49.5)
Missing	0	0	0	0	0
Education (%)					
High school or less	18 (26.5)	4 (22.2)	519 (24.3)	47 (22.0)	702 (19.7)
More than high school	41 (60.3)	11 (61.1)	1,481 (69.3)	131 (61.2)	2,786 (78.0)
Missing	9 (13.2)	3 (16.7)	136 (6.4)	36 (16.8)	85 (2.4)
Oral contraceptive use					
Ever	34 (50.0)	6 (33.3)	1,140 (53.4)	79 (36.9)	2,349 (65.7)
Never	34 (50.0)	12 (66.7)	996 (46.6)	135 (63.1)	1,224 (34.3)
Missing	0	0	0	0	0
HRT use					
Ever	7 (10.3)	3 (16.7)	355 (16.6)	40 (18.7)	747 (20.9)
Never	58 (85.3)	12 (66.7)	1,711 (80.1)	157 (73.4)	2,720 (76.1)
Missing	3 (4.4)	3 (16.7)	70 (3.3)	17 (7.9)	106 (3.0)
*BRCA*1/2 Status					
Positive	34 (50)	6 (33.3)	294 (13.8)	45 (21.0)	396 (11.1)
Negative	33 (48.5)	10 (55.6)	1,798 (84.2)	160 (74.8)	3,125 (87.5)
Missing	1 (1.5)	2 (11.1)	44 (2.1)	9 (4.2)	52 (1.5)

Table 3.2. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping, Breast Cancer Family Registry

	BR-OV		OV-BR		DPBOC		BR		OV	
Exposure	**Age-adjusted model (Cases n=68)**	**Multi-variable model* (Cases n=68)**	**Age-adjusted model (Cases n=18)**	**Multi-variable model* (Cases n=18)**	**Age-adjusted model (Cases n=86)**	**Multi-variable model* (Cases n=86)**	**Age-adjusted model (Cases n=2,136)**	**Multi-variable model* (Cases n=2,136)**	**Age-adjusted model (Cases n=214)**	**Multi-variable model* (Cases n=214)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use										
Never	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Ever	0.44 (0.25, 0.74)	0.40 (0.22, 0.69)	0.25 (0.04, 0.60)	0.30 (0.05, 0.70)	0.39 (0.23, 0.61)	0.38 (0.22, 0.60)	0.62 (0.55, 0.70)	0.63 (0.56, 0.71)	0.35 (0.23, 0.45)	0.37 (0.25, 0.48)
Parity										
Nulliparous	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
1 child	3.37 (0.65, 9.98)	3.42 (0.69, 10.01)	<0.01 (<0.01, 4.37)	<0.01 (<0.01, 3.89)	2.50 (0.57, 7.29)	2.50 (0.58, 7.56)	2.36 (1.85, 2.89)	2.28 (1.79, 2.82)	1.36 (0.69, 2.49)	1.27 (0.63, 2.35)
≥2 children	6.73 (3.01, 16.62)	6.42 (2.80, 16.42)	4.67 (1.45, >999)	3.98 (1.01, >999)	6.21 (3.16, 14.74)	5.78 (2.82, 14.58)	2.50 (2.07, 2.97)	2.40 (1.98, 2.84)	1.95 (1.19, 3.11)	1.70 (1.02, 2.68)
Age at First Birth										
Nulliparous	0.12 (0.05, 0.28)	0.13 (0.05, 0.30)	0.18 (<0.01, 0.59)	0.21 (<0.01,0.85)	0.13 (0.05, 0.25)	0.15 (0.06, 0.28)	0.41 (0.34, 0.50)	0.43 (0.36, 0.52)	0.52 (0.33, 0.88)	0.61 (0.39, 1.03)
<20 years	0.76 (0.21, 1.39)	0.73 (0.20, 1.33)	0.34 (<0.01, 1.70)	0.36 (<0.01, 1.83)	0.67 (0.21, 1.23)	0.66 (0.21, 1.20)	0.98 (0.79, 1.21)	0.98 (0.78, 1.21)	0.92 (0.48, 1.56)	0.93 (0.48, 1.60)
20-24 years	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
25-29 years	0.48 (0.24, 0.89)	0.51 (0.25, 0.97)	0.42 (<0.01, 1.35)	0.42 (<0.01,1.42)	0.46 (0.24, 0.81)	0.49 (0.25, 0.88)	1.09 (0.91, 1.24)	1.07 (0.92, 1.25)	1.08 (0.78, 1.64)	1.12 (0.81, 1.70)
>29 years	0.25 (0.05, 0.61)	0.29 (0.05, 0.72)	0.21 (<0.01,0.89)	0.23 (<0.01, 1.17)	0.24 (0.05, 0.53)	0.28 (0.05, 0.64)	0.95 (0.78, 1.11)	0.96 (0.80, 1.16)	0.66 (0.38, 1.13)	0.74 (0.42, 1.24)
Age at Last Birth										
Nulliparous	0.16 (0.06, 0.38)	0.17 (0.07, 0.40)	0.31 (<0.01,1.99)	0.37 (<0.01,3.00)	0.18 (0.08, 0.37)	0.20 (0.09, 0.40)	0.39 (0.32, 0.48)	0.41 (0.33, 0.51)	0.50 (0.30, 0.86)	0.57 (0.35, 0.99)
<25 years	0.91 (0.28, 1.86)	0.84 (0.25, 1.71)	0.60 (<0.01,5.10)	0.63 (<0.01, 5.70)	0.87 (0.29, 1.66)	0.83 (0.27, 1.59)	0.92 (0.72, 1.12)	0.90 (0.70, 1.10)	0.72 (0.33, 1.26)	0.73 (0.33, 1.28)
25-29 years	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
30-34 years	0.79 (0.42, 1.70)	0.82 (0.42, 1.78)	1.58 (0.43, 8.68)	1.62 (0.44, 9.64)	0.91 (0.54, 1.78)	0.95 (0.56, 1.87)	0.93 (0.79, 1.06)	0.95 (0.81, 1.10)	0.89 (0.60, 1.37)	0.90 (0.61, 1.39)
>34 years	0.57 (0.21, 1.42)	0.60 (0.21, 1.52)	0.99 (<0.01,6.44)	1.03 (<0.01, 7.13)	0.63 (0.29, 1.41)	0.67 (0.29, 1.50)	0.90 (0.73, 1.05)	0.94 (0.77, 1.12)	1.04 (0.66, 1.57)	1.06 (0.67, 1.62)

Breastfeed										
Never	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Ever	0.47 (0.28, 0.81)	0.49 (0.28, 0.84)	0.57 (0.18, 2.25)	0.63 (0.20, 2.74)	0.49 (0.29, 0.83)	0.52 (0.31, 0.87)	0.71 (0.64, 0.84)	0.80 (0.70, 0.93)	0.83 (0.58, 1.17)	0.89 (0.62, 1.27)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Table 3.3. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping stratified by BRCA1/2 status and age at diagnosis/interview, Breast Cancer Family Registry

***BRCA*1/2 Mutation Negative**			
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=43)**	**Multivariable model* (Cases n=1,798)**	**Multivariable model* (Cases n=160)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.21 (0.10, 0.41)	0.59 (0.52, 0.67)	0.32 (0.21, 0.46)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	2.48 (<0.01, 9.99)	2.27 (1.76, 2.90)	1.10 (0.44, 2.20)
≥2 children	3.90 (1.35, 13.14)	2.27 (1.87, 2.80)	1.66 (0.98, 2.70)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.77 (0.39, 1.77)	0.81 (0.70, 0.94)	0.80 (0.55, 1.24)
***BRCA*1/2 Mutation Positive**			
	Multivariable model* (Cases n=40)	**Multivariable model* (Cases n=294)**	**Multivariable model* (Cases n=45)**
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.82 (0.40, 1.88)	1.02 (0.69, 1.47)	0.78 (0.31, 1.73)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	2.17 (<0.01, 14.95)	2.56 (1.17, 5.36)	1.84 (0.35, 8.01)
≥2 children	7.03 (2.34, 44.16)	3.14 (1.55, 5.62)	1.25 (0.24, 4.42)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.35 (0.14, 1.09)	0.73 (0.52, 1.33)	1.18 (0.49, 4.17)
Age at Diagnosis/Interview ≤40			
	Multivariable model* (Cases n=31)	**Multivariable model* (Cases n=618)**	**Multivariable model* (Cases n=42)**
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.28 (0.13, 0.68)	0.67 (0.53, 0.85)	0.34 (0.16, 0.68)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	2.86 (<0.01, 10.67)	2.14 (1.41, 3.28)	2.67 (0.68, 7.39)
≥2 children	2.92 (0.79, 12.66)	1.94 (1.36, 2.78)	1.28 (0.39, 3.41)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.39 (0.18, 1.52)	0.64 (0.46, 0.92)	0.43 (0.16, 1.23)
Age at Diagnosis/Interview >40			
	Multivariable model* (Cases n=53)	**Multivariable model* (Cases n=1,520)**	**Multivariable model* (Cases n=172)**
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.35 (0.18, 0.57)	0.48 (0.40, 0.55)	0.32 (0.20, 0.44)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	1.07 (<0.01, 7.24)	1.54 (1.15, 2.00)	0.67 (0.25, 1.46)
≥2 children	4.16 (1.78, 21.79)	1.55 (1.22, 1.90)	1.21 (0.67, 2.22)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.51 (0.26, 0.91)	0.80 (0.69, 0.94)	0.96 (0.67, 1.44)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Chapter 4. Hormonal and Reproductive Risk Factors for Single and Double Primary Breast and Ovarian Cancer in a Cohort Enriched for Increased Familial Risk, ProF-SC

4.1. Abstract

Background: With enhancements in screening and treatment, breast cancer survivors make up the largest group of cancer survivors in the U.S. Developing a second primary cancer is one of the most severe long-term outcomes of a cancer diagnosis, particularly if the second primary cancer lacks an effective screening tool as with ovarian cancer. Limited research has been done to assess risk factors for second primary ovarian cancer following a breast cancer diagnosis and even more limited are prospective studies that minimize information and selection bias. Using data from a family-based cohort enriched for increased familial risk, we evaluated the association between hormonal and reproductive factors and risk of second primary ovarian cancer following a breast cancer diagnosis.

Methods: Using data from The Breast Cancer Prospective Family Study Cohort (ProF-SC) we evaluated the association between oral contraceptive (OC) use, number of full-term (FT) pregnancies, and breastfeeding and risk of second primary ovarian cancer following breast cancer (BR-OV), risk of first primary ovarian cancer (OV), and risk of first primary breast cancer (BR) using Cox proportional hazards. We evaluated effect measure modification by predicted lifetime risk of breast and ovarian cancer to evaluate differences in these associations across the risk spectrum.

Results: OC use was associated with a non-statistically significant greater risk of BR-OV (HR=1.62, 95% CI: 0.91, 2.90) and the risk was stronger and statistically significant in women with a lifetime risk of breast and ovarian cancer ≥20% (high risk) (HR=3.05, 95% CI: 1.02, 9.13). However, when we stratified by time between breast cancer diagnosis and the baseline interview, the positive association between OC use and BR-OV was only seen in the prevalent cancers (breast cancer diagnosis >2 years before baseline) and not in the pseudo-incident cancers (breast cancer diagnosis ≤2 years before baseline). OC use was associated with a borderline statistically significant lower risk of OV (HR=0.59, 95% CI: 0.34, 1.00) and this association was stronger in women at high risk (HR=0.33, 95% CI: 0.13, 0.86). OC use was not associated with BR overall but when we stratified by an attained age of 52 years we observed a greater

risk in older women (HR=1.39, 95% CI: 1.11, 1.73). Having two or more FT pregnancies was associated with a lower risk of BR-OV (HR=0.47, 95% CI: 0.22, 0.97). While number of FT pregnancies was not associated with OV or BR, later age at first pregnancy was associated with an increased risk of OV for women aged 25-29 years versus <20 years (HR=3.51, 95% CI: 1.08, 11.40) and an increased risk of BR for women aged ≥30 years versus <20 years (HR=1.49, 95% CI: 1.09, 2.04). We observed no association between breastfeeding and any of our outcomes.

Conclusion: This study suggests that OC use may have a discordant effect on ovarian cancer depending on whether it is a first primary or a second primary cancer, and on underlying risk of breast or ovarian cancer. Being able to identify potentially modifiable risk factors for second primary cancers is critical for cancer survivors and women at increased risk of breast and ovarian cancer.

4.2. Introduction

Improvements in the detection and treatment of cancer have led to an increasing number of people surviving a cancer diagnosis. According to the American Cancer Society, as of January 1, 2016 there were more than 15.5 million children and adults living with a history of cancer. More than half this group (67%) was diagnosed 5 or more years ago and 17% were diagnosed 20 or more years ago. By January 2026 it is estimated that there will be 20.3 million cancer survivors [176]. According to SEER, cancer survivors have a 14% increased risk of developing a second primary cancer compared to the general population [177]. With the growing population of cancer survivors, research is needed to better understand the etiology of second primary cancers and to identify potential means of prevention.

Many factors may contribute to the development of second primary cancers including shared risk factors, shared genetics, and treatment of the first cancer. Breast and ovarian cancer share multiple risk factors (e.g., parity and breastfeeding), and genetics (e.g. *BRCA*1/2), and have been found to co-occur [34-41]. The bi-directional nature of primary and secondary breast and ovarian cancer suggests that shared risk factors and genetics, rather than treatment, are the main contributors of this association [86]. Developing a second primary cancer is a serious adverse event for cancer survivors and research has shown that women with breast cancer who develop a second primary cancer have worse survival [178,179]. Research into double primary breast and ovarian cancer (DPBOC) has shown that diagnosis order matters in terms of survival. Liou and colleagues found that women with ovarian cancer following breast cancer (BR-OV) had worse survival than women with breast cancer following ovarian cancer (OV-BR). Contributing to this finding, ovarian cancers in the BR-OV group were higher stage, higher grade, and more likely to be serous versus other histologic subtypes, compared to ovarian cancers in the OV-BR group [171]. High-grade serous carcinoma is the most common type of ovarian cancer and has the worst survival [180].

Few studies have examined potentially modifiable risk factors for DPBOC; one prospective cohort study examining risk of BR-OV [86] and three retrospective case-control studies, assessing OV-BR [87], DPBOC combined [88], and BR-OV and OV-BR (Chapter 3). While retrospective case-control studies are advantageous for identifying a larger number of cases, something particularly important for rare events

such as second primary cancers, they are susceptible to information and selection bias. Recall bias is a concern in case-control studies as recall may be influenced by disease status; a minimal concern for exposures such as parity and breastfeeding, which are major life events, but possibly a greater concern for exposures such as oral contraceptive (OC) use. In addition, selection bias is a concern in case-control studies with the selection of controls, particularly if the source population is difficult to define. Prospective cohort studies, on the other hand, avoid many forms of information and selection bias but identifying a sufficient number of cases may be a challenge. Therefore, prospective studies which are potentially more valid are less efficient. In order to address these gaps we conducted a prospective cohort study using a family-based cohort of breast and ovarian cancer families enriched for increased familial risk to examine the association between OC use, parity, and breastfeeding and three outcomes: 1) second primary ovarian cancer following a breast cancer diagnosis (BR-OV), 2) single primary ovarian cancer (OV), and 3) single primary breast cancer (BR).

4.3. Methods

We conducted a prospective cohort study using participants from The Breast Cancer Prospective Family Study Cohort (ProF-SC) who were enrolled in the six sites of the Breast Cancer Family Registry (BCFR). ProF-SC has been described in detail elsewhere [181]. Briefly, ProF-SC includes all female participants from the BCFR [126] who were enrolled before June 30, 2011. There are three population-based sites (Australia, Northern California, and Ontario) and three clinic-based sites (New York, Philadelphia, and Ontario). The three population-based sites recruited case families through cancer registries and control families via random digit dialing in the same catchment area as the local cancer registry. Australia over-sampled cases with an early age at diagnosis whereas Northern California and Ontario over-sampled cases with an early age at diagnosis and/or having a family history or other genetic predisposition. The three clinic-based sites enrolled case families through local hospitals, organizations, and breast cancer support groups. Further details of recruitment at the three clinic-based sites are described in Chapter 3.

Exposure information: Starting in 1996 the BCFR administered baseline epidemiologic questionnaires to collect data on demographics, reproductive factors, medical history, family history, and

other behavioral factors such as alcohol consumption and cigarette smoking. Beginning in 2007 the BCFR initiated a follow-up study to update information on vital status, personal and family history of cancer, and breast cancer risk factors that were collected at baseline. The BCFR has sought biospecimen collection on all cases and blood or buccal samples on most participants. Screening for *BRCA*1 and *BRCA*2 germline mutations was conducted on the proband or the youngest affected family member and if a deleterious mutation was found then screening was performed on family members. In order to assess underlying risk of breast and ovarian cancer in participants included in this analysis, we obtained predicted breast and ovarian cancer risk scores using the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) [138]. We stratified by lifetime risk of breast or ovarian cancer ≥20% versus <20% as this cutpoint has been used by the National Comprehensive Cancer Network to identify women at high risk [21]. As a sensitivity analysis we used the continuous measure of predicted 1-year risk of breast or ovarian cancer.

Outcome information: For our double primary cancer groups, the first primary cancer was prevalent (cancer diagnosed prior to baseline interview) and the second primary cancer was incident (cancer diagnosed after the baseline interview). The single primary groups were incident cancers. Figure 4.1 displays the exclusion criteria and sample size for our four initial analytic groups. For the affected breast group (followed prospectively for BR-OV) and unaffected group (followed prospectively for OV) we excluded women with a prior bilateral oophorectomy, and for the affected ovarian group (followed prospectively for OV-BR) and unaffected group (followed prospectively for BR) we excluded women with a prior bilateral mastectomy. For all of the groups we excluded women who were 80 years of age or older at the baseline interview and anyone missing the age at censor variable. Our final sample sizes were as follows: 1) affected breast group: n = 8,969, 2) affected ovarian group: n = 298, 3) Unaffected group (followed for OV): n = 11,870, 4) Unaffected group (followed for BR): n = 12,974. Cancer confirmation was sought on all cases. The Australia, Ontario, and Utah sites of the BCFR regularly link to local population-based cancer registries to confirm cases at their sites and all BCFR sites link to the National Death Index or other death registries to update vital status. Additionally, pathology and medical reports were sought for all cases. While cancer confirmation is ongoing, we have confirmation of both cancers for our BR-OV cases on 31 (45.6%) and of at least one cancer on 61 (89.7%). We have confirmation in

32 (47.1%) of our OV cases, and 559 (74.7%) of our BR cases. Cancer treatment was self-reported using a validated questionnaire that collected information on cancer stage and type of initial breast or ovarian treatment (e.g. surgery, chemotherapy, radiotherapy). We currently have breast treatment data on 53 (77.9%) of our BR-OV cases.

4.3.1. Statistical Analysis

While we initially established four analytic groups of interest: 1) affected breast followed for ovarian (BR-OV), 2) affected ovarian followed for breast (OV-BR), 3) unaffected followed for ovarian (OV), and 4) unaffected followed for breast (BR), we only observed 2 cases of OV-BR and were unable to examine this group in a multivariable model. Therefore, we only present the baseline data for the OV-BR group. We examined mean and percent differences between baseline measures of our covariates of interest and our three main exposures (OC use, parity, and breastfeeding) for each of our four initial analytic groups. As we had data at two time points (baseline and follow-up) we created time-varying covariates for our exposures and covariates of interest that could change over time using a simple update method. If a value was only available at one time point then that value was used for the entire study period. The variables for height and BMI at baseline and follow-up were evaluated and outliers were removed in the following two-step process: 1) Heights greater than three standard deviations away from the mean were removed (n = 75 (1 BR case) for baseline, n = 33 (1 case) for follow-up) and 2) BMIs greater than 50kg/m^2 and less than or equal to 14kg/m^2 were removed (n = 72 (2 BR cases) for baseline, n = 31 (0 cases) for follow-up). In order to test the robustness of our height and BMI variables we ran analyses with the cleaned time-varying covariates, the original time-varying covariates, and the baseline variables. As we observed minimal differences in our results with these three sets of height and BMI variables, we used the cleaned time-varying covariates in our final models. We used Cox proportional hazards models to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the association between OC use, parity, and breastfeeding and risk of BR-OV, OV, and BR using age as the time-scale. Person-time was calculated differently for the three analytic groups we were able to examine in a multivariable model. For the BR-OV group we calculated person-time from age at breast cancer diagnosis until the earliest of the following ages: age at ovarian cancer diagnosis, age at bilateral prophylactic oophorectomy, age at death, age at last follow-up, or age 80 years. For the OV group we

calculated person-time from age at the baseline interview until the earliest of the following ages: age at ovarian cancer diagnosis, age at bilateral prophylactic oophorectomy, age at death, age at last follow-up, or age 80 years. For the BR group we calculated person-time from age at the baseline interview until the earliest of the following ages: age at breast cancer diagnosis, age at bilateral prophylactic mastectomy, age at death, age at last follow-up, or age 80 years. We tested the proportional hazards assumption for our main exposures using an interaction between our main exposures and the natural log of age and by examining the cumulative sums of martingale residuals over attained age [182]. We used a robust sandwich estimator to account for the family-based nature of the cohort. We developed directed acyclic graphs (DAGs) to identify confounders of our proposed causal effects. Additionally, we assessed the following variables for confounding using the 10% change-in-beta criterion: race/ethnicity (white vs. non-white), education level (>high school degree vs. ≤high school degree), cigarette use (ever vs. never), alcohol consumption (ever vs. never), age at menarche (years), menopausal status (pre- vs. post-menopausal), hormone replacement therapy use (ever vs. never), height (meters), and BMI (kg/m^2). If a covariate was identified as a potential confounder using either the DAG method or the statistical method then it was included in our multivariable model. Based on these two approaches all of the covariates were included in our multivariable models. We stratified our models by birth cohort (<1950, 1950-1959, 1960-1969, ≥1970) to account for secular changes in our exposures and outcomes over time. We dealt with missing covariate data (less than 3% for all covariates) by including a missing indicator variable for those covariates with missing data. We conducted a sensitivity analysis where individuals missing covariate data were excluded from the multivariable models and observed minimal differences in our results; therefore we included the missing indicators to maximize our statistical power. To test the robustness of our Cox proportional hazards model we ran parallel models using Poisson regression.

In order to evaluate whether the associations between our main exposures and outcomes varied by predicted risk of breast and ovarian cancer, we examined effect measure modification (interaction) on the multiplicative and additive scale. Interaction on the multiplicative scale was assessed by including an interaction term in our multivariable models between our main exposures and predicted risk and using either the Wald Test of the Likelihood Ratio Test. Interaction on the additive scale was determined using the relative excess risk due to interaction (RERI).

The following sensitivity analyses were performed to test the robustness of our results: 1) Multivariable models using baseline variables rather than time-varying covariates (Supplemental Table 4.1); 2) Exposures truncated to the age of diagnosis of the first primary breast cancer for the BR-OV group (Supplemental Table 4.2); 3) Excluding *in situ* breast cancers for the BR-OV and BR groups (Supplemental Table 4.3). We observed minimal differences in our results across sensitivity analyses and therefore retained our time-varying covariates and included all cancers to enhance our statistical power.

All analyses were conducted using SAS version 9.4.

4.4. Results

Table 4.1a reports baseline differences in our exposed and unexposed groups for OC use, parity, and breastfeeding by our covariates of interest for the BR-OV group. In this group, 0.48% were missing OC use, 0.06% were missing parity, and 0.36% were missing breastfeeding data. Compared to never OC users, ever OC users were more likely to be younger at baseline, pre-menopausal, to have ever smoked cigarettes, and to have ever consumed alcohol. Compared to nulliparous women, parous women were more likely to be post-menopausal, to have never consumed alcohol, to have a high school degree or less, and to have been born before 1950. Compared to parous women who never breastfed, parous women who ever breastfed were more likely to be from the Australia site, pre-menopausal, and to have been born in 1950 or later.

Table 4.1b reports baseline differences for the OV-BR group. In this group, 1.34% were missing OC use, 0.00% were missing parity, and 0.34% were missing breastfeeding data. Compared to never OC users, ever OC users were more likely to be from the Philadelphia site but less likely to be from the New York site, more likely to have ever smoked cigarettes, to have ever consumed alcohol, to have more than a high school degree, to have ever used HRT, and to be born in 1950 or later. Compared to nulliparous women, parous women were more likely to have a high school degree or less, and to have been born before 1950. Compared to parous women who never breastfed, parous women who ever breastfed were more likely to be from the Utah site, non-white, to have never smoked cigarettes, to have never used HRT, and to have been born in 1950 or later.

Table 4.1c reports baseline differences for the OV group. In this group, 0.51% were missing OC use, 0.03% were missing parity, and 0.76% were missing breastfeeding data. Compared to never OC users, ever OC users were younger at baseline, more likely to be pre-menopausal, to have ever smoked cigarettes, to have ever consumed alcohol, to have more than a high school degree, and to be born in 1950 or later. Compared to nulliparous women, parous women were older at baseline and at censor, more likely to be post-menopausal, to have a high school degree or less, to have ever used HRT, to have been born before 1950, and to have a higher BMI. Compared to parous women who never breastfed, parous women who ever breastfed were more likely to be from the Australia site, pre-menopausal, to have more than a high school degree, and to have been born in 1950 or later.

Table 4.1d reports baseline differences for the BR group. In this group, 0.49% were missing OC use, 0.03% were missing parity, and 0.77% were missing breastfeeding data. Compared to never OC users, ever OC users were younger at baseline, more likely to be pre-menopausal, to have ever smoked cigarettes, to have ever consumed alcohol, to have more than a high school degree, and to be born in 1950 or later. Compared to nulliparous women, parous women were older at baseline and at censor, more likely to be post-menopausal, to have a high school degree or less, to have ever used HRT, to have been born before 1950, and to have a higher BMI. Compared to parous women who never breastfed, parous women who ever breastfed were more likely to be from the Australia site, pre-menopausal, to have more than a high school degree, and to have been born in 1950 or later.

Table 4.2 reports the multivariable results of our Cox proportional hazards regression analyses for the BR-OV, OV, and BR groups. We observed a non-statistically significant greater risk of BR-OV for ever OC use versus never use (HR=1.62, 95% CI: 0.91, 2.90) but a borderline statistically significant lower risk of OV (HR=0.59, 95% CI: 0.34, 1.00). There was no association between OC use and risk of BR; however, the proportional hazards assumption was violated for OC use in this group. Therefore, we stratified by an attained age of 52 years after review of the cumulative distribution of the martingale residuals. For those with an attained age greater than 52 years, ever OC use was associated with an increased risk of BR (HR=1.31, 95% CI: 1.05, 1.65). For those with an attained age less than or equal to 52 years, there was no association between OC use and risk of BR (HR=0.87, 95% CI: 0.64, 1.18). For

number of full-term (FT) pregnancies, we observed a statistically significant lower risk of BR-OV for those with two or more FT pregnancies compared to nulliparous women (HR=0.47, 95% CI: 0.22, 0.97). We observed a statistically significant lower risk of BR in our crude model (HR=0.77, 95% CI: 0.61, 0.98); however the association became attenuated and no longer statistically significant after adjustment for potential confounders. There was no association between number of FT pregnancies and risk of OV. We found no association between later age at first FT pregnancy and risk of BR-OV. In contrast, we observed a statistically significant greater risk of OV for women with a first FT pregnancy 25-29 years compared to less than 20 years (HR=3.51, 95% CI: 1.08, 11.40) and a statistically significant greater risk of BR for women with an age at first FT pregnancy 30 years or later compared to less than 20 years (HR=1.49, 95% CI: 1.09, 2.04). We found no association between years since last FT pregnancy and risk of BR-OV, OV, and BR. However, the proportional hazards assumption was violated for this variable in the OV group so we stratified by an attained age of 52 years based on the cumulative distribution of the martingale residuals. While we did not observe any statistically significant associations between years since last FT pregnancy and risk of OV, there was a suggestion of a greater risk in younger women and a lower risk in older women for 0-5 years since last FT pregnancy versus nulliparous (data not shown). Lastly, we observed no association between breastfeeding or breastfeeding and pregnancy combined and BR-OV, OV, and BR. In order to test the robustness of our results we ran Poisson regression and compared these results to our main findings using Cox proportional hazards (Supplemental Table 4.4). We observed minimal differences in the results of the two models suggesting our findings are robust to model specification.

Figures 4.2a, 4.2b, and 4.2c show our multivariable results stratified by lifetime risk of breast or ovarian cancer greater than or equal to 20% (high risk) compared to less than 20% (average risk) for OC use, number of FT pregnancies, and breastfeeding, respectively. For ever versus never OC use we observed a greater risk of BR-OV in those at high risk (HR=3.05, 95% CI: 1.02, 9.13) but this finding was attenuated and not statistically significant for those at average risk (HR=1.21, 95% CI: 0.60, 2.43). In contrast, for ever OC use we observed a lower risk of OV in those at high risk (HR=0.33, 95% CI: 0.13, 0.86); however, this finding was attenuated and not statistically significant in those at average risk (HR=0.72, 95% CI: 0.38, 1.38). Overall there was no association between OC use and risk of BR in

women at high or average risk, but when we stratified by attained age 52 years to account for the proportional hazards violation there was a suggestion of a greater risk in older women in the high risk group (HR=1.54, 95% CI: 0.79, 3.01), but not in younger women in the high risk group (HR=1.07, 95% CI: 0.70, 1.63). Overall we found no association between number of FT pregnancies and risk of BR-OV, OV, and BR in either the high or average risk groups; however there was a borderline inverse association between having 2 or more FT pregnancies and BR-OV in the average risk group (HR=0.46, 95% CI: 0.20, 1.08). Lastly, there was no association between breastfeeding and risk of BR-OV, OV, and BR when we stratified by lifetime risk. Despite these differences in our risk factor associations between women at high and average risk, we did not observe any statistically significant interactions on either the multiplicative or additive scale for lifetime risk.

In order to determine whether our exposures had a differential effect on risk of first primary ovarian cancer (OV) versus second primary ovarian cancer (BR-OV), we combined our OV and BR-OV analytic groups and assessed interaction on the multiplicative and additive scale between our exposures and pre-baseline breast cancer status. We observed a borderline statistically significant multiplicative interaction between OC use and pre-baseline breast cancer status (p=0.07) but no additive interaction. When we stratified by lifetime risk we observed a statistically significant multiplicative interaction in women at high risk between OC use and pre-baseline breast cancer status (p<0.05) and breastfeeding and pre-baseline breast cancer status (p<0.05), but no additive interaction.

We further examined our results stratified by *BRCA*1/2 mutation status (Supplemental Table 4.5). Due to a small number of events in the *BRCA*1/2 mutation positive stratum in the OV group we were unable to obtain multivariable estimates. In the *BRCA*1/2 mutation positive group, there was a suggestion of a greater risk of BR-OV for ever OC users versus never users (HR=2.10, 95% CI: 0.80, 5.48), however it was not statistically significant. Overall there was no association between OC use and risk of BR in either *BRCA*1/2 mutation group, but when we stratified by attained age 52 years there was a statistically significant greater risk for older women in both *BRCA*1/2 mutation positive and negative groups (HR=3.10, 95% CI: 1.04, 9.25) and (HR= 1.27, 95% CI: 1.01, 1.61), respectively. We observed similar non-statistically significant lower risks of BR-OV for 1 and ≥2 FT pregnancies for both *BRCA*1/2

positive and negative groups; however they did not reach statistical significance. Similarly, in the *BRCA*1/2 mutation negative group there was an inverse association between one FT pregnancy versus nulliparous and OV, however the results were not statistically significant. In contrast, for *BRCA*1/2 positive women we observed a statistically significant positive association between having 1 FT pregnancy versus nulliparous and risk of BR (HR=2.66, 95% CI: 1.19, 5.91), but no association in *BRCA*1/2 negative women (multiplicative interaction $p<0.05$).

In order to evaluate survivor bias in the BR-OV group we conducted a sensitivity analysis stratifying by breast cancers diagnosed within 2 years of the baseline interview (pseudo-incident cancers) and breast cancers diagnosed more than 2 years from the baseline interview (prevalent cancers) (Supplemental Table 4.6). The positive association between OC use and risk of BR-OV was only seen in the prevalent breast cancers and there was no association between OC use and risk of BR-OV in the pseudo-incident breast cancers.

4.5. Discussion

In this prospective cohort study of breast cancer families representing women across the risk spectrum, we examined the association between hormonal and reproductive risk factors and the risk of BR-OV, OV, and BR. For women who had ever used OCs compared to women who had never used OCs, we observed a non-statistically significant greater risk of BR-OV. Further, when we stratified by lifetime risk of breast and ovarian cancer, this association became stronger and statistically significant in women at high risk compared to women at average risk. In contrast, we observed an inverse association between OC use and risk of OV. When we stratified by lifetime risk of breast or ovarian cancer this association only remained in the high risk group. These findings suggest that OC use may have a differential effect on ovarian cancer risk depending on whether it is a first primary or second primary cancer, and whether a woman is at high or average risk. We observed a positive association between OC use and BR but this association was limited to women with an attained age of 52 years or more. Further, when we stratified by lifetime risk this association appeared stronger in the high risk group.

Our finding of an increased risk of BR-OV with ever OC use contradicts a study by Trentham-Diaz and colleagues which found no association between OC use and risk of BR-OV; however they had a

limited number of events (n=36) in their study. Additionally, this result contradicts our case-control findings of an inverse association between OC use and BR-OV. It is possible that there were differences between the BR-OV cases from our case-control study compared to this cohort study that could have contributed to these discordant OC use findings. We had limited tumor characteristic data for our cases, particularly in the case-control study, so we were unable to investigate whether differences in tumor histology or molecular characteristics contributed to the observed differences. Two studies have observed differences in the association between OC use and ovarian cancer by histology and molecular characteristics with one study showing a non-statistically significant greater risk for mucinous and clear cell cancers [183] and one study showing no association with mucinous cancers and with well-differentiated serous cancers [184]. While the median age at breast cancer diagnosis was the same between studies (44 years), the median time between the breast and ovarian cancer diagnoses was 7 years in the case-control study and 11 years in this cohort study, suggesting possible differences in participant or tumor characteristics. Lastly, differences in study design could have contributed to these discordant findings as retrospective case-control studies are more susceptible to recall and selection bias compared to prospective cohort studies. However, survivor bias may have affected the results of our case-control and cohort study as both studies included pseudo-incident cancers (diagnosed ≤2 years before baseline) and prevalent cancers (diagnosed >2 years before baseline). When the cohort study was restricted to the pseudo-incident cancers, we observed no association between OC use and BR-OV. In order to explore this finding, we examined baseline differences between the prevalent and pseudo-incident cases. Women with prevalent first primary breast cancer were more likely to be older, post-menopausal, to have ever smoked cigarettes, to have ever consumed alcohol, to have been born before 1950, and to be at high risk (*BRCA*1/2 mutation carriers and ≥20% lifetime risk of breast or ovarian cancer). It is possible that women born before 1950 were exposed to OCs with higher estrogen formulations compared to women born after 1950 which could have contributed to the discrepant findings; however all of our models were stratified by birth cohort. Further, our multivariable models were adjusted for menopausal status, cigarette smoking, and alcohol consumption. When we stratified by risk based on *BRCA*1/2 mutation status or BOADICEA lifetime risk, the positive association was only seen in the high risk groups which corresponds with the finding of more high risk women in the prevalent versus incident

cancers. So it is possible that the positive association between OC use and BR-OV in high risk women was partially driven by there being more prevalent cancers in this group. Lastly, it is possible that information bias may have contributed to the discrepant OC use findings between prevalent and pseudo-incident cases. Parity and breastfeeding, which are major life events, may be easier to recall than OC use, and their findings were not discordant between the prevalent and pseudo-incident cases. Recall of OC use, on the other hand, may have been less accurate for the prevalent cases as they were older and more likely to be post-menopausal; however, OC use has been shown to be reliably recalled [174,175] and using a crude ever versus never categorization of OC use should improve recall.

Our finding of an inverse association between OC use and OV is consistent with the literature (reviewed in [45]). Additionally, our finding of an increased risk of BR in older women is consistent with much of the literature (reviewed in [45]) and some studies have similarly observed differences in the association between OC use and BR by age of breast cancer diagnosis with some studies showing higher risks [145,185] and one study reporting a lower risk [186] in women diagnosed at older ages. While our models were stratified by birth cohort, a possible explanation of our finding of an increased risk of BR in older women could be related to differences in OC formulations over time. A study by Work and colleagues observed an increased risk of ER/PR negative breast cancer with OC use before 1975 but not in 1975 or later [5]. While we were missing ER/PR data on 55.9% of our BR cases, we ran a sensitivity analysis stratifying by known ER status and our results suggested a positive association between OC use and ER negative BR, but not ER positive BR, regardless of attained age; however the results were not statistically significant (data not shown).

We observed a lower risk of BR-OV with two or more FT pregnancies which is consistent with the study by Trentham-Diaz and colleagues which showed a non-statistically significant lower risk of BR-OV with four or more children [86], but not consistent with Cvelbar and colleagues who observed no association with DPBOC [88], or our case-control study showing an increased risk of BR-OV with two or more children (Chapter 3). We found no association between parity and OV or BR in our multivariable models which is not consistent with the literature showing a protective effect of parity on risk of OV and BR (reviewed in [45]). Further, our case-control study found an increased risk of BR which remained

throughout sensitivity analyses. We did not observe an association between age at first FT pregnancy and BR-OV. While the study by Trentham-Diaz and colleagues suggested a positive association between older age at first FT pregnancy and BR-OV, their finding was not statistically significant [86]. Older age at first FT pregnancy was associated with an increased risk of OV and BR. While these results are consistent with the breast cancer literature, the ovarian cancer literature has been mixed (reviewed in [45]). Further, we observed no association between age at first FT pregnancy and OV and BR in our case-control study.

We found no association between breastfeeding or pregnancy and breastfeeding combined with BR-OV, OV, or BR. While this null finding for BR-OV is consistent with the study by Cvelbar and colleagues [88], our findings for OV and BR are not consistent with the literature suggesting a protective effect of breastfeeding on OV [187,188] and BR (reviewed in [45]). Additionally, these results for BR-OV and BR are not consistent with our case-control study which observed an inverse association between breastfeeding and BR-OV and BR.

In order to test the robustness of our predicted measure of lifetime risk of breast and ovarian cancer using a 20% cutoff, we conducted sensitivity analyses using a continuous measure of predicted 1-year risk. While our results were similar between the two risk measures there were a few differences worth noting. While we did not observe interaction on either the multiplicative or additive scale for the association between OC use and BR-OV by lifetime risk, we observed a borderline statistically significant multiplicative interaction using the continuous measure of 1-year risk ($p=0.06$), but no additive interaction. In addition, while we did not observe interaction on either the multiplicative or additive scale for the association between OC use and BR by lifetime risk and attained age 52 years, we observed multiplicative interaction by 1-year risk ($p<0.05$), but no additive interaction.

Our study had several limitations including a limited number of OV-BR cases which prevented us from examining risk factor associations with this group and comparing to the BR-OV group to assess the importance of diagnosis order. However, our case-control study observed similar risk factor associations for the BR-OV and OV-BR groups. Information bias may have contributed to our observed findings due to missing treatment and cancer confirmation data, misclassification of our exposures, and recall bias in

the affected cohort. We had limited treatment data on our BR-OV cases (22.1% missing) to evaluate interaction between our main exposures and treatment. We had 46 BR-OV events with any chemotherapy, radiotherapy, or hormone therapy and when we examined our results limited to these cases we observed minimal differences in our results. We only had 7 BR-OV events with surgery only or no treatment and were therefore unable to examine this stratum. However, since women with breast cancer have an increased risk of ovarian cancer and women with ovarian cancer have an increased risk of breast cancer, it is unlikely that treatment is the predominant cause [86]. We had a limited number of confirmed BR-OV (45.6%) and OV (47.1%) cases and were unable to assess our results limited to confirmed cases. There could be some concern that a non-confirmed ovarian cancer following a breast cancer diagnosis is a metastasis rather than a second primary cancer, however this has been shown to be an unlikely event [169,170]. To further address this concern we ran our analyses removing synchronous cancers diagnosed within 1 year of each other (n=2) and observed minimal differences in our results with the exception being the positive association between OC use and BR-OV became stronger and statistically significant (HR = 1.93, 95% CI:1.06, 3.51). We may have had information bias through misclassification of our exposures; however, parity and breastfeeding, which are major life events, are less susceptible to misclassification and have been shown to be reliably reported [172,173]. Further, we used crude measures of these variables (ever versus never for breastfeeding and nulliparous versus parous defined as 1 or ≥2 for parity) which minimizes exposure misclassification. OC use, on the other hand, may be more subject to misclassification compared to breastfeeding and parity. However, OC use has also been shown to be reliably reported [174,175], and we similarly used a crude measure (ever versus never) which would have minimized any misclassification. For the affected cohort, exposure information was collected after their breast cancer diagnosis whereas for the unaffected cohort, exposure information was collected prior to a cancer diagnosis; therefore recall bias in the affected cohort could have influenced the results. Selection bias may have influenced our results through loss to follow-up. In the overall ProF-SC cohort 18.1% have been lost to follow-up; however those lost to follow-up had similar baseline proportions of OC use and parity as the total cohort [181]. Therefore, any misclassification due to loss to follow-up would likely be non-differential. While our cohort contained women across the risk spectrum of breast and ovarian cancer, our results may not be generalizable to breast cancer survivors

without a family history (sporadic cases) or the general population of women at average risk. Lastly, we were likely underpowered to assess interaction on both the multiplicative and additive scale in the BR-OV and OV groups. For example, while we did not find a statistically significant interaction by lifetime risk for the association between OC use and OV on either the multiplicative or additive scale, we would expect interaction on one scale as both OC use and lifetime risk were statistically significantly associated with risk of OV.

Our study also had many strengths. The BCFR recruited probands with breast or ovarian cancer and their relatives in the mid-1990s, thus establishing a family-based cohort representing women across the continuum of breast and ovarian cancer risk. Therefore, this study was uniquely able to examine established risk factors for breast and ovarian cancer in women across the spectrum of risk. This is particularly critical as studies have already shown differences in breast and ovarian cancer risk factors between average and high risk women [189,190], including our case-control study. ProF-SC was then established to follow these women prospectively extending the cohort and allowing for updated exposure and outcome information and alternative analytic designs. Further, having breast and ovarian cancer cases recruited at baseline allowed us to follow these women prospectively for the development of a second primary cancer. Therefore, we were able examine differences in risk factor associations between first primary and second primary ovarian cancer in the same study. Our study had extensive risk factor data which is often limited in large population-based registries, insurance claims databases, and clinic databases. Lastly, having data at two time points allowed us to create time-varying covariates to more accurately account for changes in covariates over time.

In the U.S. there are over 3.5 million breast cancer survivors; the largest group of cancer survivors in the country [176]. As developing a second primary cancer is one of the most severe sequelae of a cancer diagnosis, identifying cancer survivors at greatest risk is critical. While there is no established screening tool for ovarian cancer, identifying women with a breast cancer diagnosis at greatest risk of developing a second primary ovarian cancer could help inform clinical recommendations for prophylactic surgery. Our study contributes to the sparse epidemiologic literature on risk of second primary ovarian cancer following breast cancer and highlights the potential contribution of established

breast and ovarian cancer risk factors. As many of our findings were not consistent between our case-control and cohort studies regarding risk of BR-OV, additional studies are needed to replicate our findings. In particular, as oral contraceptives also function as a chemopreventive agent often prescribed to women at high risk of ovarian cancer, further work is needed to understand whether they similarly decrease or possibly increase the risk of a second primary ovarian cancer following a breast cancer diagnosis.

4.6. Tables and Figures

Figure 4.1. Exclusion criteria and sample size flow diagram for our four initial analytic groups

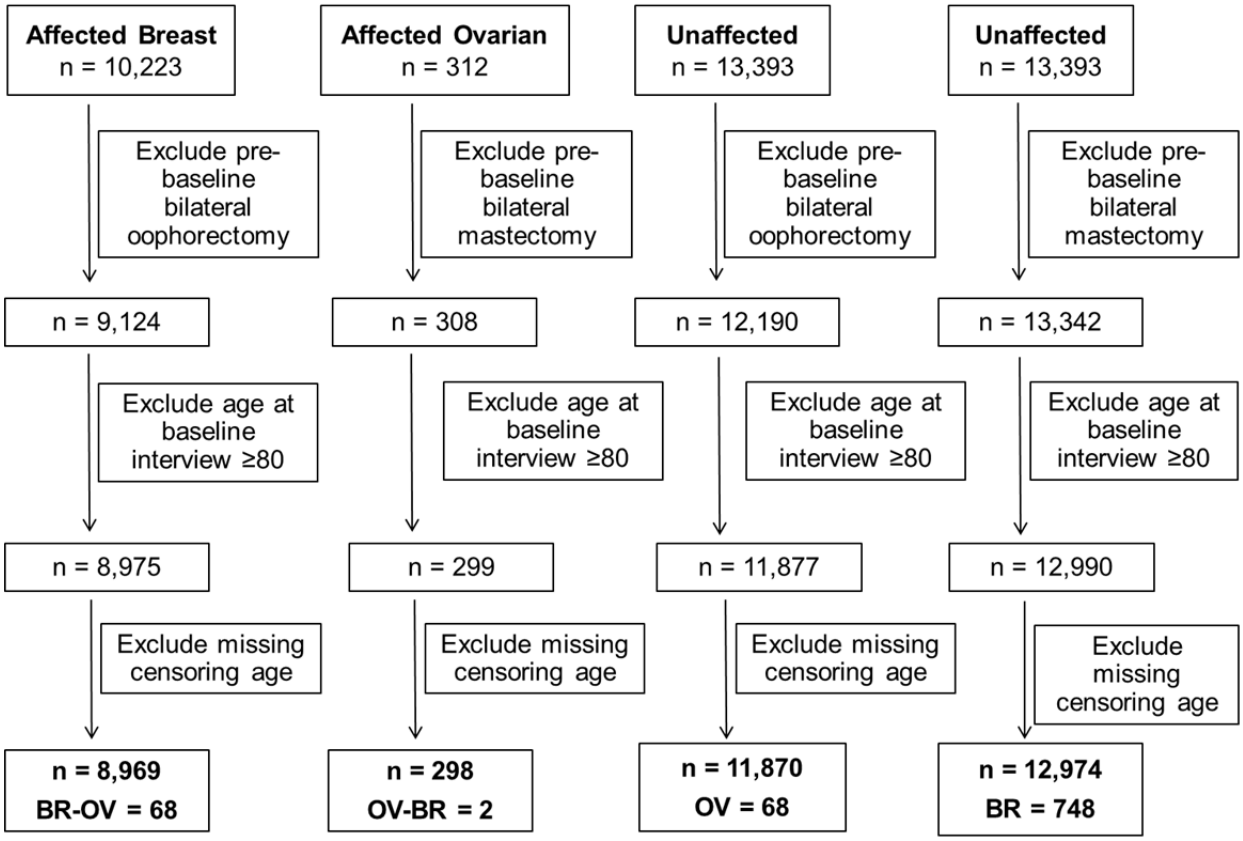


Table 4.1a. Demographic, reproductive, and behavioral differences between exposed and unexposed for the BR-OV group, ProF-SC (n = 8,969)

	Total			Ever OC Use		Never OC Use			Parous		Nulliparous			Parous, Ever Breastfed		Parous, Never Breastfed	
	N/Mean	%/SD		N/Mean	%/SD	N/Mean	%/SD		N/Mean	%/SD	N/Mean	%/SD		N/Mean	%/SD	N/Mean	%/SD
Age at Baseline	50.63	10.95		48.84	9.93	55.06	12.01		51.57	10.78	46.90	10.83		50.50	10.91	53.95	10.04
Age at Censure	58.23	12.06		56.81	11.42	61.79	12.78		59.05	11.96	54.97	11.89		58.11	12.08	61.14	11.42
Study Site																	
Philadelphia	546	6.09		339	5.34	186	7.21		434	6.06	110	6.11		250	5.09	169	7.62
New York	904	10.08		543	8.56	355	13.76		709	9.90	194	10.77		420	8.55	289	13.04
Utah	188	2.10		130	2.05	57	2.21		169	2.36	19	1.05		135	2.75	33	1.49
Australia	1615	18.01		1326	20.90	288	11.16		1295	18.08	320	17.77		1090	22.18	202	9.11
Ontario	1906	21.25		1374	21.65	522	20.23		1541	21.51	365	20.27		1020	20.76	514	23.18
California	3810	42.48		2634	41.51	1172	45.43		3015	42.09	793	44.03		1999	40.68	1010	45.56
Race																	
White	5485	61.16		4090	64.45	1376	53.33		4350	60.73	1133	62.91		3075	62.58	1255	56.61
Other	3444	38.40		2238	35.27	1190	46.12		2789	38.94	652	36.20		1826	37.16	951	42.90
Missing	40	0.45		18	0.28	14	0.54		24	0.34	16	0.89		13	0.26	11	0.50
Menopausal Status																	
Pre	3768	42.01		2985	47.04	763	29.57		2821	39.38	945	52.47		2140	43.55	665	30.00
Post	5201	57.99		3361	52.96	1817	70.43		4342	60.62	856	47.53		2774	56.45	1552	70.00
Cigarette Use																	
Ever	3741	41.71		2889	45.52	844	32.71		3014	42.08	727	40.37		1957	39.82	1041	46.96
Never	5188	57.84		3439	54.19	1728	66.98		4139	57.78	1045	58.02		2952	60.07	1173	52.91
Missing	40	0.45		18	0.28	8	0.31		10	0.14	29	1.61		5	0.10	3	0.14
Alcohol Use																	
Ever	3837	42.78		3045	47.98	786	30.47		2927	40.86	908	50.42		2008	40.86	911	41.09
Never	5077	56.61		3270	51.53	1785	69.19		4216	58.86	860	47.75		2901	59.04	1293	58.32
Missing	55	0.61		31	0.49	9	0.35		20	0.28	33	1.83		5	0.10	13	0.59
Education																	

> High school	6137	68.42		4512	71.10	1612	62.48		4667	65.15	1467	81.45		3388	68.95	1262	56.92
≤ High school	2778	30.97		1809	28.51	954	36.98		2468	34.45	309	17.16		1507	30.67	946	42.67
Missing	54	0.60		25	0.39	14	0.54		28	0.39	25	1.39		19	0.39	9	0.41
HRT Use																	
Ever	2001	22.31		1441	22.71	556	21.55		1672	23.34	328	18.21		1059	21.55	611	27.56
Never	6861	76.50		4855	76.50	1987	77.02		5434	75.86	1425	79.12		3826	77.86	1587	71.58
Missing	107	1.19		50	0.79	37	1.43		57	0.80	48	2.67		29	0.59	19	0.86
Year of birth																	
<1950	4463	49.76		2859	45.05	1584	61.40		3769	52.62	691	38.37		2354	47.90	1398	63.06
1950 - 1959	2788	31.08		2132	33.60	649	25.16		2202	30.74	585	32.48		1619	32.95	578	26.07
1960 - 1969	1477	16.47		1176	18.53	290	11.24		1056	14.74	420	23.32		832	16.93	215	9.70
≥1970	241	2.69		179	2.82	57	2.21		136	1.90	105	5.83		109	2.22	26	1.17
***BRCA*1/2 Status**																	
Positive	595	6.63		448	7.06	146	5.66		468	6.53	127	7.05		339	6.90	126	5.68
Negative	8374	93.37		5898	92.94	2434	94.34		6695	93.47	1674	92.95		4575	93.10	2091	94.32
Age at Menarche	12.68	1.58		12.66	1.56	12.74	1.64		12.69	1.58	12.67	1.62		12.69	1.56	12.67	1.61
Missing	122	1.36		51	0.80	51	1.98		88	1.23	32	1.78		51	1.04	35	1.58
BMI, kg/m2	26.03	5.67		25.97	5.68	26.17	5.66		26.27	5.68	25.05	5.54		25.95	5.43	26.95	6.13
Missing	234	2.61		139	2.19	85	3.29		186	2.60	46	2.55		127	2.58	56	2.53
Height, M	1.63	0.07		1.63	0.07	1.61	0.07		1.62	0.07	1.63	0.07		1.63	0.07	1.62	0.07
Missing	95	1.06		56	0.88	33	1.28		75	1.05	19	1.05		51	1.04	21	0.95

Table 4.1b. Demographic, reproductive, and behavioral differences between exposed and unexposed for the OV-BR group, ProF-SC (n = 298)

	Total			Ever OC Use		Never OC Use			Parous		Nulliparous			Parous, Ever Breastfed		Parous, Never Breastfed	
	N/Mean	%/SD		N/Mean	%/SD	N/Mean	%/SD		N/Mean	%/SD	N/Mean	%/SD		N/Mean	%/SD	N/Mean	%/SD
Age at Baseline	54.53	11.86		51.23	10.52	58.28	12.33		55.68	11.70	49.44	11.29		54.71	12.23	57.33	10.71
Age at Censure	63.09	12.48		59.76	11.49	66.96	12.62		64.13	12.40	58.51	11.88		63.31	12.78	65.62	11.67
Study Site																	
Philadelphia	46	15.44		32	20.38	13	9.49		36	14.81	10	18.18		17	11.33	18	19.57
New York	161	54.03		70	44.59	89	64.96		128	52.67	33	60.00		75	50.00	53	57.61
Utah	31	10.40		22	14.01	9	6.57		28	11.52	3	5.45		25	16.67	3	3.26
Australia	15	5.03		7	4.46	7	5.11		14	5.76	1	1.82		10	6.67	4	4.35
Ontario	13	4.36		9	5.73	4	2.92		11	4.53	2	3.64		7	4.67	4	4.35
California	32	10.74		17	10.83	15	10.95		26	10.70	6	10.91		16	10.67	10	10.87
Race																	
White	214	71.81		118	75.16	94	68.61		172	70.78	42	76.36		100	66.67	72	78.26
Other	80	26.85		37	23.57	42	30.66		68	27.98	12	21.82		48	32.00	19	20.65
Missing	4	1.34		2	1.27	1	0.73		3	1.23	1	1.82		2	1.33	1	1.09
Menopausal Status																	
Pre	25	8.39		16	10.19	9	6.57		17	7.00	8	14.55		14	9.33	3	3.26
Post	273	91.61		141	89.81	128	93.43		226	93.00	47	85.45		136	90.67	89	96.74
Cigarette Use																	
Ever	122	40.94		75	47.77	47	34.31		98	40.33	24	43.64		55	36.67	43	46.74
Never	174	58.39		81	51.59	90	65.69		144	59.26	30	54.55		94	62.67	49	53.26
Missing	2	0.67		1	0.64	.	.		1	0.41	1	1.82		1	0.67	.	.
Alcohol Use																	
Ever	102	34.23		63	40.13	38	27.74		83	34.16	19	34.55		52	34.67	31	33.70
Never	192	64.43		93	59.24	97	70.80		158	65.02	34	61.82		97	64.67	61	66.30
Missing	4	1.34		1	0.64	2	1.46		2	0.82	2	3.64		1	0.67	.	.
Education																	

> High school	211	70.81		122	77.71	87	63.50		165	67.90	46	83.64		105	70.00	59	64.13
≤ High school	84	28.19		33	21.02	50	36.50		76	31.28	8	14.55		44	29.33	32	34.78
Missing	3	1.01		2	1.27	.	.		2	0.82	1	1.82		1	0.67	1	1.09
HRT Use																	
Ever	169	56.71		100	63.69	69	50.36		141	58.02	28	50.91		77	51.33	64	69.57
Never	124	41.61		56	35.67	66	48.18		99	40.74	25	45.45		71	47.33	28	30.43
Missing	5	1.68		1	0.64	2	1.46		3	1.23	2	3.64		2	1.33	.	.
Year of birth																	
<1950	187	62.75		83	52.87	102	74.45		161	66.26	26	47.27		92	61.33	68	73.91
1950 - 1959	73	24.50		52	33.12	19	13.87		55	22.63	18	32.73		37	24.67	18	19.57
1960 - 1969	29	9.73		15	9.55	14	10.22		21	8.64	8	14.55		16	10.67	5	5.43
≥1970	9	3.02		7	4.46	2	1.46		6	2.47	3	5.45		5	3.33	1	1.09
***BRCA*1/2 Status**																	
Positive	60	20.13		33	21.02	26	18.98		51	20.99	9	16.36		34	22.67	17	18.48
Negative	238	79.87		124	78.98	111	81.02		192	79.01	46	83.64		116	77.33	75	81.52
Age at Menarche	12.49	1.57		12.45	1.66	12.54	1.47		12.46	1.61	12.61	1.37		12.62	1.70	12.23	1.43
Missing	5	1.68		2	1.27	1	0.73		4	1.65	1	1.82		2	1.33	2	2.17
BMI, kg/m2	26.35	6.12		26.19	6.63	26.55	5.56		26.42	5.63	26.06	7.98		26.28	5.54	26.66	5.82
Missing	5	1.68		3	1.91	1	0.73		4	1.65	1	1.82		3	2.00	1	1.09
Height, M	1.62	0.06		1.63	0.06	1.61	0.06		1.62	0.06	1.63	0.07		1.62	0.06	1.62	0.06
Missing	2	0.67		1	0.64	0	0		2	0.82	0	0		2	1.33	0	0

Table 4.1c. Demographic, reproductive, and behavioral differences between exposed and unexposed for the OV group, ProF-SC (n = 11,870)

	Total			Ever OC Use		Never OC Use			Parous		Nulliparous			Parous, Ever Breastfed		Parous, Never Breastfed	
	N/Mean	%/SD		N/Mean	%/SD	N/Mean	%/SD		N/Mean	%/SD	N/Mean	%/SD		N/Mean	%/SD	N/Mean	%/SD
Age at Baseline	45.93	15.00		43.31	12.97	52.91	17.64		49.93	13.46	34.37	13.11		49.10	13.63	52.88	12.46
Age at Censure	56.67	14.84		54.62	13.45	62.19	16.83		60.55	13.16	45.46	13.65		59.92	13.33	62.83	12.31
Study Site																	
Philadelphia	826	6.96		614	7.13	193	6.03		586	6.65	239	7.83		347	5.19	175	8.60
New York	1810	15.25		1157	13.44	639	19.96		1172	13.30	638	20.90		826	12.35	346	16.99
Utah	693	5.84		536	6.23	155	4.84		483	5.48	210	6.88		435	6.51	45	2.21
Australia	3756	31.64		2767	32.15	975	30.45		2801	31.78	954	31.25		2438	36.46	349	17.14
Ontario	2210	18.62		1713	19.90	492	15.37		1613	18.30	597	19.55		1156	17.29	450	22.10
California	2575	21.69		1820	21.15	748	23.36		2158	24.49	415	13.59		1485	22.21	671	32.96
Race																	
White	8807	74.20		6580	76.45	2196	68.58		6353	72.09	2452	80.31		4948	73.99	1319	64.78
Other	2972	25.04		1978	22.98	979	30.57		2403	27.27	567	18.57		1697	25.38	702	34.48
Missing	91	0.77		49	0.57	27	0.84		57	0.65	34	1.11		42	0.63	15	0.74
Menopausal Status																	
Pre	7740	65.21		6258	72.71	1442	45.03		5084	57.69	2655	86.96		4068	60.83	948	46.56
Post	4130	34.79		2349	27.29	1760	54.97		3729	42.31	398	13.04		2619	39.17	1088	53.44
Cigarette Use																	
Ever	4775	40.23		3710	43.10	1052	32.85		3690	41.87	1083	35.47		2671	39.94	986	48.43
Never	7025	59.18		4860	56.47	2137	66.74		5096	57.82	1928	63.15		4009	59.95	1048	51.47
Missing	70	0.59		37	0.43	13	0.41		27	0.31	42	1.38		7	0.10	2	0.10
Alcohol Use																	
Ever	5365	45.20		4288	49.82	1065	33.26		3818	43.32	1545	50.61		2961	44.28	826	40.57
Never	6412	54.02		4262	49.52	2120	66.21		4949	56.16	1462	47.89		3710	55.48	1200	58.94
Missing	93	0.78		57	0.66	17	0.53		46	0.52	46	1.51		16	0.24	10	0.49
Education																	

> High school	8019	67.56		6195	71.98	1798	56.15		5446	61.80	2570	84.18		4335	64.83	1049	51.52
≤ High school	3779	31.84		2377	27.62	1384	43.22		3330	37.79	448	14.67		2328	34.81	975	47.89
Missing	72	0.61		35	0.41	20	0.62		37	0.42	35	1.15		24	0.36	12	0.59
HRT Use																	
Ever	2187	18.42		1565	18.18	612	19.11		1950	22.13	237	7.76		1366	20.43	581	28.54
Never	9450	79.61		6895	80.11	2535	79.17		6717	76.22	2731	89.45		5262	78.69	1432	70.33
Missing	233	1.96		147	1.71	55	1.72		146	1.66	85	2.78		59	0.88	23	1.13
Year of birth																	
<1950	4460	37.57		2637	30.64	1796	56.09		4050	45.95	408	13.36		2863	42.81	1159	56.93
1950 - 1959	2889	24.34		2312	26.86	564	17.61		2392	27.14	496	16.25		1845	27.59	508	24.95
1960 - 1969	2509	21.14		2119	24.62	381	11.90		1781	20.21	728	23.85		1470	21.98	295	14.49
≥1970	2012	16.95		1539	17.88	461	14.40		590	6.69	1421	46.54		509	7.61	74	3.63
***BRCA*1/2 Status**																	
Positive	558	4.70		414	4.81	140	4.37		367	4.16	190	6.22		302	4.52	56	2.75
Negative	11312	95.30		8193	95.19	3062	95.63		8446	95.84	2863	93.78		6385	95.48	1980	97.25
Age at Menarche	12.86	1.58		12.82	1.55	12.95	1.63		12.88	1.59	12.78	1.55		12.89	1.56	12.87	1.67
Missing	182	1.53		110	1.28	37	1.16		107	1.21	74	2.42		81	1.21	24	1.18
BMI, kg/m2	25.63	5.64		25.57	5.67	25.77	5.55		26.10	5.62	24.28	5.48		25.83	5.39	26.95	6.21
Missing	282	2.38		194	2.25	74	2.31		205	2.33	75	2.46		147	2.20	54	2.65
Height, M	1.63	0.07		1.64	0.07	1.62	0.07		1.63	0.07	1.65	0.07		1.63	0.07	1.62	0.07
Missing	125	1.05		83	0.96	31	0.97		91	1.03	34	1.11		63	0.94	25	1.23

Table 4.1d. Demographic, reproductive, and behavioral differences between exposed and unexposed groups for the BR group, ProF-SC (n = 12,974)

	Total			Ever OC Use		Never OC Use			Parous		Nulliparous			Parous, Ever Breastfed		Parous, Never Breastfed	
	N/Mean	%/SD		N/Mean	%/SD	N/Mean	%/SD		N/Mean	%/SD	N/Mean	%/SD		N/Mean	%/SD	N/Mean	%/SD
Age at Baseline	47.06	15.13		44.21	13.16	54.29	17.28		50.91	13.49	35.19	13.66		50.03	13.72	53.85	12.34
Age at Censor	57.74	14.85		55.47	13.51	63.53	16.42		61.46	13.08	46.25	14.06		60.79	13.30	63.77	12.08
Study Site																	
Philadelphia	909	7.01		666	7.19	224	6.14		653	6.67	255	8.03		376	5.14	206	8.67
New York	1900	14.64		1198	12.93	687	18.84		1247	12.73	653	20.56		872	11.92	375	15.78
Utah	787	6.07		604	6.52	181	4.96		570	5.82	217	6.83		511	6.98	56	2.36
Australia	4003	30.85		2902	31.33	1086	29.78		3028	30.92	974	30.67		2627	35.90	387	16.28
Ontario	2405	18.54		1817	19.61	583	15.99		1786	18.24	619	19.49		1263	17.26	514	21.62
California	2970	22.89		2077	22.42	886	24.29		2510	25.63	458	14.42		1668	22.80	839	35.30
Race																	
White	9569	73.76		7029	75.87	2508	68.77		7031	71.79	2536	79.85		5405	73.87	1531	64.41
Other	3310	25.51		2184	23.58	1110	30.44		2703	27.60	605	19.05		1868	25.53	830	34.92
Missing	95	0.73		51	0.55	29	0.80		60	0.61	35	1.10		44	0.60	16	0.67
Menopausal Status																	
Pre	7764	59.84		6276	67.75	1447	39.68		5104	52.11	2659	83.72		4080	55.76	951	40.01
Post	5210	40.16		2988	32.25	2200	60.32		4690	47.89	517	16.28		3237	44.24	1426	59.99
Cigarette Use																	
Ever	5246	40.43		4016	43.35	1216	33.34		4113	42.00	1131	35.61		2927	40.00	1150	48.38
Never	7651	58.97		5204	56.17	2418	66.30		5649	57.68	2001	63.00		4380	59.86	1225	51.54
Missing	77	0.59		44	0.47	13	0.36		32	0.33	44	1.39		10	0.14	2	0.08
Alcohol Use																	
Ever	5789	44.62		4553	49.15	1224	33.56		4187	42.75	1600	50.38		3198	43.71	953	40.09
Never	7083	54.59		4647	50.16	2404	65.92		5554	56.71	1528	48.11		4100	56.03	1412	59.40
Missing	102	0.79		64	0.69	19	0.52		53	0.54	48	1.51		19	0.26	12	0.50
Education																	
> High school	8611	66.37		6588	71.11	1996	54.73		5950	60.75	2658	83.69		4670	63.82	1211	50.95

≤ High school	4286	33.04		2639	28.49	1628	44.64		3803	38.83	482	15.18		2620	35.81	1153	48.51
Missing	77	0.59		37	0.40	23	0.63		41	0.42	36	1.13		27	0.37	13	0.55
HRT Use																	
Ever	3030	23.35		2117	22.85	902	24.73		2703	27.60	327	10.30		1848	25.26	851	35.80
Never	9692	74.70		6987	75.42	2684	73.59		6929	70.75	2761	86.93		5404	73.86	1499	63.06
Missing	252	1.94		160	1.73	61	1.67		162	1.65	88	2.77		65	0.89	27	1.14
Year of birth																	
<1950	5300	40.85		3088	33.33	2184	59.88		4811	49.12	487	15.33		3356	45.87	1425	59.95
1950 - 1959	3098	23.88		2471	26.67	614	16.84		2566	26.20	531	16.72		1947	26.61	574	24.15
1960 - 1969	2562	19.75		2164	23.36	388	10.64		1826	18.64	736	23.17		1505	20.57	303	12.75
≥1970	2014	15.52		1541	16.63	461	12.64		591	6.03	1422	44.77		509	6.96	75	3.16
BRCA1/2 Status																	
Positive	619	4.77		458	4.94	157	4.30		424	4.33	194	6.11		345	4.72	67	2.82
Negative	12355	95.23		8806	95.06	3490	95.70		9370	95.67	2982	93.89		6972	95.28	2310	97.18
Age at Menarche	12.85	1.58		12.81	1.56	12.96	1.64		12.88	1.59	12.78	1.56		12.88	1.57	12.87	1.67
Missing	191	1.47		114	1.23	40	1.10		113	1.15	77	2.42		84	1.15	27	1.14
BMI, kg/m2	25.82	5.68		25.75	5.71	25.98	5.59		26.28	5.66	24.40	5.52		25.99	5.44	27.13	6.18
Missing	299	2.30		202	2.18	83	2.28		220	2.25	77	2.42		159	2.17	57	2.40
Height, M	1.63	0.07		1.64	0.07	1.62	0.07		1.62	0.07	1.65	0.07		1.63	0.07	1.62	0.07
Missing	131	1.01		85	0.92	35	0.96		95	0.97	36	1.13		66	0.90	26	1.09

Table 4.2. Hormonal and reproductive factors and risk of BR-OV, OV, and BR using Cox proportional hazards, ProF-SC

	BR-OV			OV			BR		
		Model 1*	Model 2**		Model 1*	Model 2**		Model 1*	Model 2**
Exposure	No. Events	Events (n = 68) Person-time*** (111,152 py)	Events (n = 68) Person-time*** (111,152 py)	No. Events	Events (n = 68) Person-time*** (133,712 py)	Events (n = 68) Person-time*** (133,712 py)	No. Events	Events (n = 748) Person-time*** (145,052)	Events (n = 748) Person-time*** (145,052)
		HR (95% CI)	HR (95% CI)		HR (95% CI)	HR (95% CI)		HR (95% CI)	HR (95% CI)
Oral Contraceptive Use									
Never	17	1.00 (Ref)	1.00 (Ref)	24	1.00 (Ref)	1.00 (Ref)	182	1.00 (Ref)	1.00 (Ref)
Ever	51	1.33 (0.75, 2.36)	1.62 (0.91, 2.90)	44	0.66 (0.39, 1.12)	0.59 (0.34, 1.00)	566	1.17 (0.97, 1.41)	1.14 (0.95, 1.38)
Number of FT Pregnancies									
Nulliparous	18	1.00 (Ref)	1.00 (Ref)	14	1.00 (Ref)	1.00 (Ref)	145	1.00 (Ref)	1.00 (Ref)
1	10	0.63 (0.25, 1.55)	0.64 (0.26, 1.62)	4	0.46 (0.15, 1.43)	0.56 (0.17, 1.81)	88	0.89 (0.66, 1.19)	0.98 (0.72, 1.32)
≥2	40	0.50 (0.24, 1.05)	0.47 (0.22, 0.97)	50	0.78 (0.35, 1.74)	1.02 (0.44, 2.38)	515	0.77 (0.61, 0.98)	0.88 (0.68, 1.13)
Age at 1st FT Pregnancy									
Nulliparous	18	2.00 (0.81, 4.98)	1.95 (0.79, 4.81)	14	3.88 (0.93, 16.19)	1.98 (0.50, 7.83)	145	1.56 (1.15, 2.12)	1.35 (0.98, 1.87)
<20	7	1.00 (Ref)	1.00 (Ref)	3	1.00 (Ref)	1.00 (Ref)	81	1.00 (Ref)	1.00 (Ref)
20 - 24	20	1.19 (0.50, 2.83)	1.10 (0.48, 2.51)	29	3.70 (1.15, 11.96)	2.94 (0.68, 12.78)	243	1.26 (0.98, 1.61)	1.22 (0.94, 1.58)
25 - 29	13	0.90 (0.36, 2.28)	0.80 (0.32, 2.02)	16	3.17 (0.95, 10.60)	3.51 (1.08, 11.40)	164	1.28 (0.98, 1.66)	1.20 (0.91, 1.60)
≥30	10	1.11 (0.42, 2.93)	1.05 (0.42, 2.63)	6	2.13 (0.56, 8.09)	2.87 (0.84, 9.77)	115	1.59 (1.19, 2.12)	1.49 (1.09, 2.04)
Year Since Last FT Pregnancy									
Nulliparous	18	2.08 (0.98, 4.43)	2.17 (1.02, 4.63)	14	1.39 (0.62, 3.10)	1.09 (0.48, 2.46)	145	1.21 (0.94, 1.56)	1.06 (0.81, 1.38)
0 - 5	1	1.02 (0.12, 8.69)	1.00 (0.11, 8.69)	2	1.56 (0.29, 8.46)	1.75 (0.29, 10.51)	20	1.03 (0.62, 1.70)	1.01 (0.61, 1.68)
6 - 20	16	1.44 (0.71, 2.91)	1.38 (0.68, 2.80)	12	0.89 (0.41, 1.93)	0.90 (0.41, 1.98)	156	0.88 (0.68, 1.15)	0.86 (0.66, 1.12)
≥21	33	1.00 (Ref)	1.00 (Ref)	40	1.00 (Ref)	1.00 (Ref)	427	1.00 (Ref)	1.00 (Ref)
Breastfeeding									
Never	31	1.00 (Ref)	1.00 (Ref)	27	1.00 (Ref)	1.00 (Ref)	288	1.00 (Ref)	1.00 (Ref)
Ever	37	1.34 (0.71, 2.52)	1.37 (0.74, 2.56)	41	1.01 (0.54, 1.90)	1.05 (0.56, 1.98)	460	1.07 (0.88, 1.29)	1.05 (0.86, 1.27)

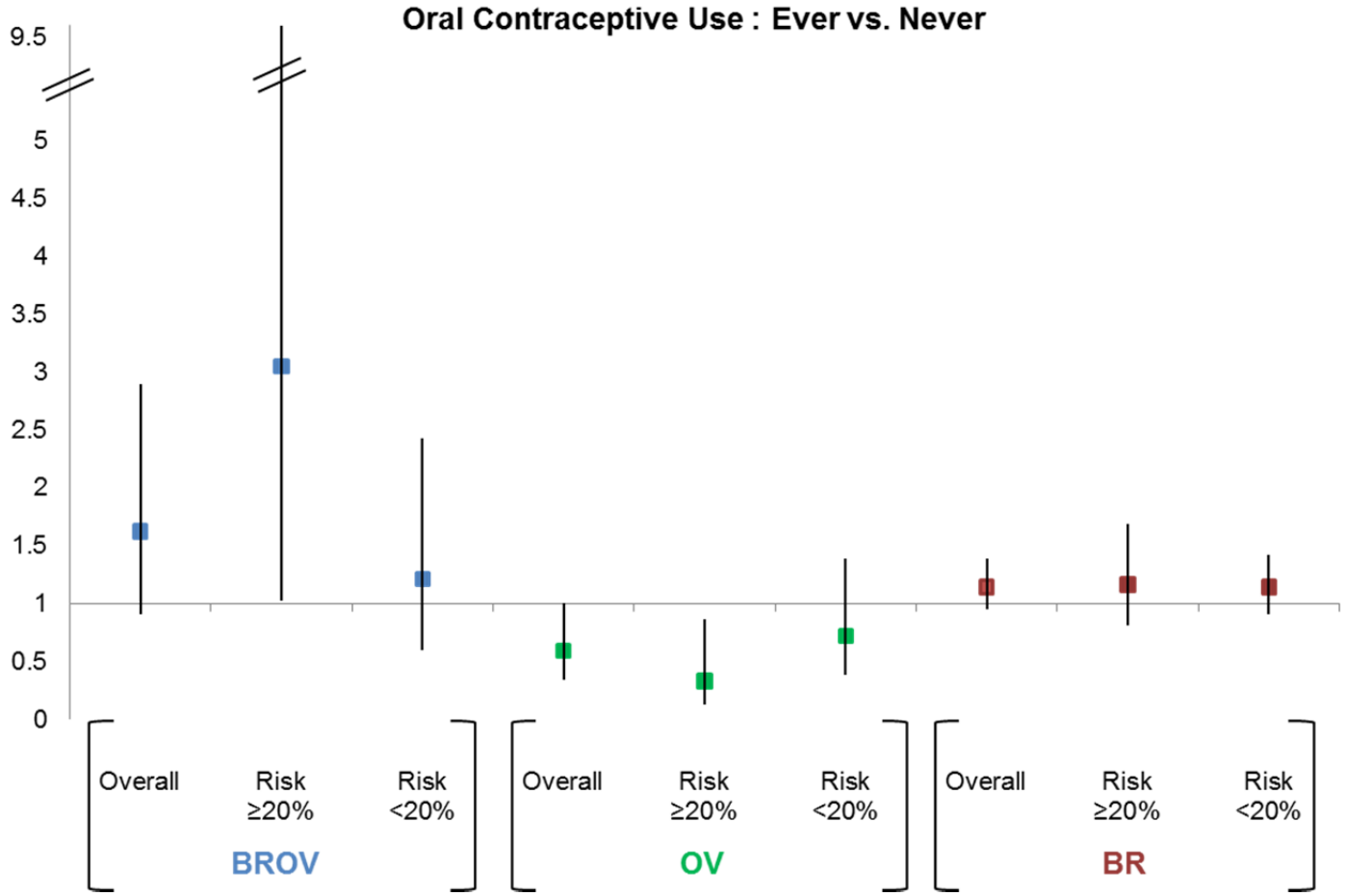
Number of FT Pregnancies & Breastfeeding									
Nulliparous	18	1.00 (Ref)	1.00 (Ref)	14	1.00 (Ref)	1.00 (Ref)	145	1.00 (Ref)	1.00 (Ref)
Any FTP/Never Breastfed	13	0.53 (0.26, 1.10)	0.51 (0.24, 1.05)	13	0.72 (0.33, 1.55)	0.92 (0.41, 2.08)	143	0.80 (0.63, 1.01)	0.90 (0.71, 1.15)
Any FTP/Ever Breastfed	37	0.70 (0.40, 1.24)	0.68 (0.38, 1.22)	41	0.75 (0.40, 1.39)	1.00 (0.52, 1.92)	460	0.84 (0.69, 1.02)	0.93 (0.76, 1.14)

*Regression models conditional on birth cohort; hazard ratios include all main exposures (OC use, number of FT pregnancies, and breastfeeding)

**Regression models conditional on birth cohort; hazard ratios adjusted for study center, race/ethnicity, age at menarche, menopausal status, BMI, height, education, cigarette use, alcohol consumption, hormone replacement therapy, and all main exposures

***Age is the time-scale

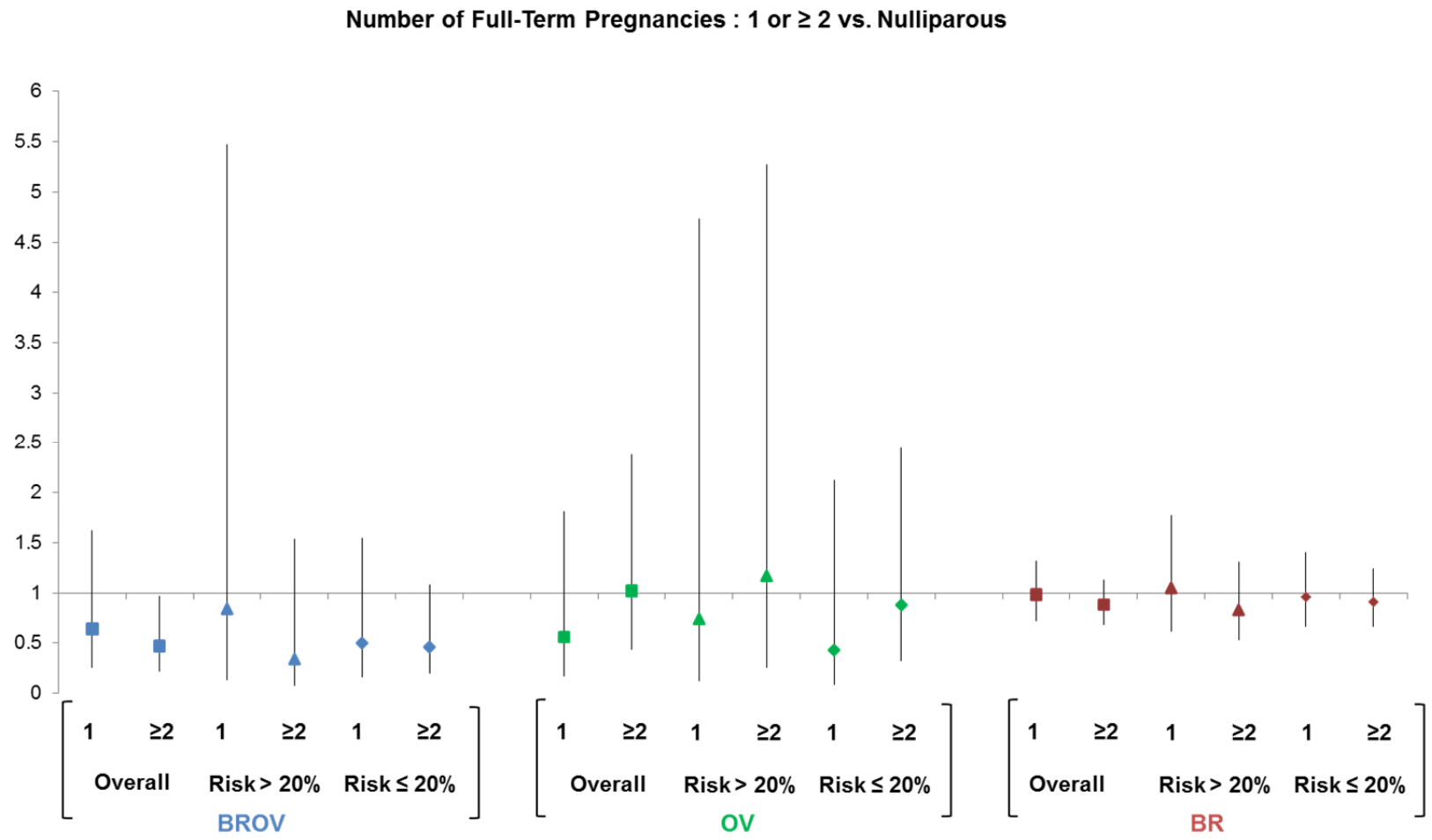
Figure 4.2a. Oral contraceptive use and risk of BR-OV, OV, and BR by risk profile, ProF-SC



*Regression models conditional on birth cohort; hazard ratios adjusted for study center, race/ethnicity, age at menarche, menopausal status, BMI, height, education, cigarette use, alcohol consumption, hormone replacement therapy, and all main exposures

**Age is the time-scale

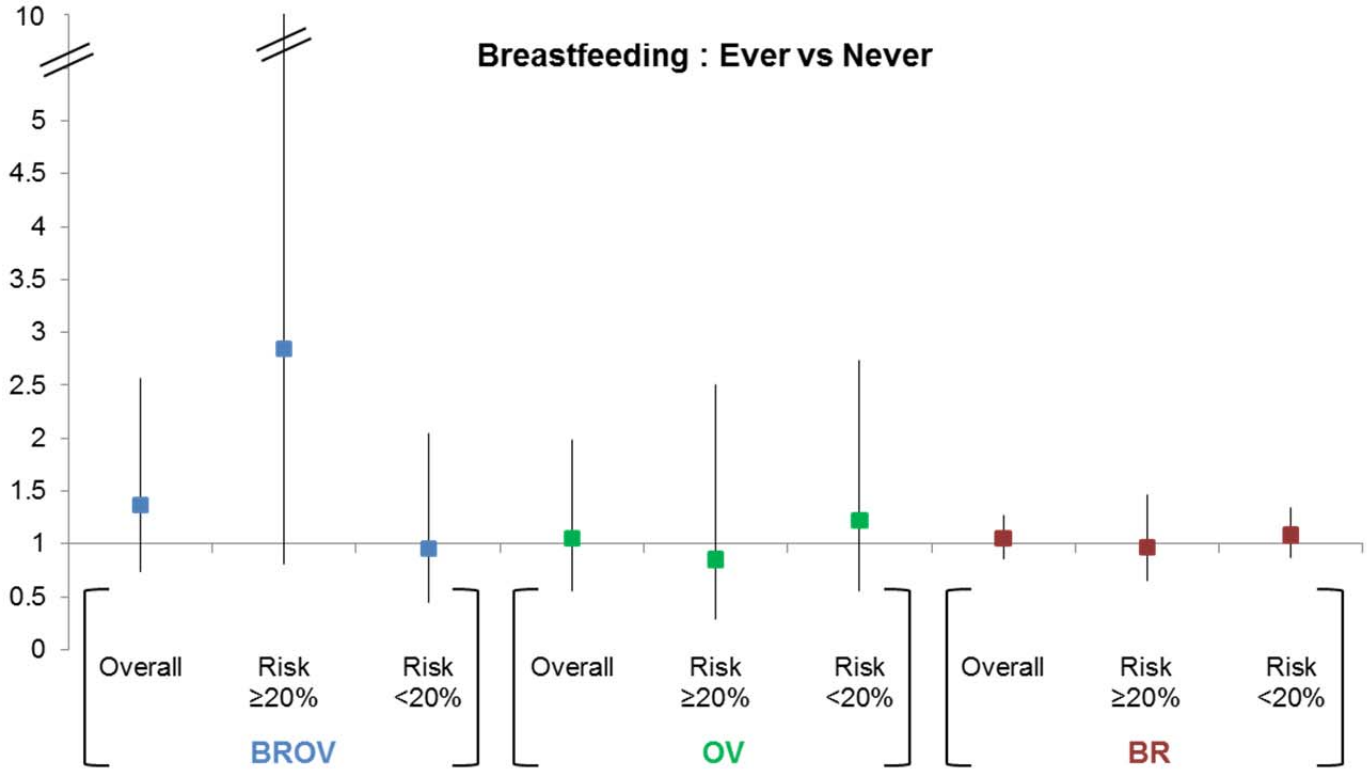
Figure 4.2b. Number of full-term pregnancies and risk of BR-OV, OV, and BR by risk profile, ProF-SC



*Regression models conditional on birth cohort; hazard ratios adjusted for study center, race/ethnicity, age at menarche, menopausal status, BMI, height, education, cigarette use, alcohol consumption, hormone replacement therapy, and all main exposures

**Age is the time-scale

Figure 4.2c. Breastfeeding and risk of BR-OV, OV, and BR by risk profile, ProF-SC



*Regression models conditional on birth cohort; hazard ratios adjusted for study center, race/ethnicity, age at menarche, menopausal status, BMI, height, education, cigarette use, alcohol consumption, hormone replacement therapy, and all main exposures

**Age time-scale

Chapter 5. Methodological Considerations in the Study of Double Primary Breast and Ovarian Cancer

In 1889 Dr. Theodore Billroth first described cases of multiple primary tumors in patients with skin cancer without metastases who later developed cancers of the stomach and bowel [191,192]. Since that first report, cases of multiple primary tumors were increasingly documented and in 1932 Warren and Gates developed the following criteria for multiple malignancies which are generally still followed today: 1) each tumor must be malignant, 2) each tumor must be distinct, and 3) one tumor cannot be the metastasis of the other [193-195]. Over time, research moved from identification of multiple primary tumors to understanding their etiology and research has suggested the following groups of factors to be associated with the development of second primary cancers including 1) treatment of the first cancer, 2) shared genetics, 3) shared risk factors, and 4) interactions between these factors. Breast and ovarian cancer have shared risk factors and genetics and have been found to co-occur [34-41]. As of January 1, 2016, there were approximately 3.5 million breast cancer survivors living in the U.S., the largest group of cancer survivors in the country [176]. Individuals with cancer are at an increased risk of developing a second primary cancer compared to individuals without cancer [196] and developing a second primary cancer increases the morbidity and mortality of cancer survivors, particularly when there is no effective screening tool to detect the second cancer at an early stage as with ovarian cancer. Identifying potentially modifiable risk factors for double primary breast and ovarian cancer (DPBOC) could have a large impact on the health of cancer survivors and women at increased risk of breast and ovarian cancer. Therefore, the overall goals of this dissertation were to summarize the literature on DPBOC and to evaluate the contribution of shared risk factors, genetics/underlying risk, and their interaction, to the development of DPBOC, while considering possible treatment effects. However, understanding the etiology of DPBOC is challenging due to the complexity of second cancers and methodological limitations of epidemiological studies. This chapter summarizes the results of my dissertation in light of these complexities and methodological concerns.

Chapter 2 was a comprehensive review of the literature to identify studies assessing risk factors for DPBOC. The review focused on 1) epidemiologic risk factors (yielding 18 studies), 2) genetic risk

factors (yielding 13 studies), and 3) epigenetic risk factors (yielding 0 studies). The first part of this comprehensive review assessed epidemiologic risk factors and DPBOC. The majority of studies identified assessed the role of treatment in the development of DPBOC. Only three studies were identified that assessed the association between oral contraceptive (OC) use, parity, or breastfeeding and risk of DPBOC; two were case-control studies [87,88] and one was a cohort study [86]. However, these studies were limited due to power (small number of events in each study ranging from 31 to 72 DPBOC cases) and assessment of crude associations only [88], and comparison across studies was difficult due to the use of different outcomes (ovarian cancer following breast cancer (BR-OV) [86], breast cancer following ovarian cancer (OV-BR) [87], and DPBOC combined [88]) and control groups (women with single primary breast cancer (BR) [86] and women with single primary ovarian cancer (OV) [87,88]). It is important to note that none of the studies observed any statistically significant associations between OC use, parity, or breastfeeding and their outcomes which may have been due to limited power. Results from this portion of the comprehensive review highlighted the need for more studies assessing the association between OC use, parity, and breastfeeding and risk of DPBOC. The second part of this comprehensive review assessed genetics and risk of DPBOC and most of the studies identified focused on *BRCA*1/2 mutations or family history. While differences in study design and outcome definitions made direct comparison across studies challenging, all studies reported an association between mutations in *BRCA*1/2 and risk of DPBOC [90,102-111,197]. Similarly, the majority of the studies identified supported an association between family history and risk of DPBOC [86,87,112,113]; however most only considered a dichotomous variable representing family history or no family history and did not take degree of the relative into account. Results from this portion of the comprehensive review showed that *BRCA*1/2 and family history play a role in the development of DPBOC and these variables should be considered in epidemiologic studies of DPBOC. The third part of the comprehensive review did not identify any studies assessing DNA methylation and risk of DPBOC, revealing the need for research in this area. Overall, results from this comprehensive review not only highlight the dearth of research on risk factors for DPBOC, but also how differences in outcome (diagnosis order), study design, and small sample size may contribute to discordant or null findings.

To address these gaps we conducted a case-control study (Chapter 3), where we had more cases of DPBOC and could examine associations by diagnosis order, and a prospective cohort study (Chapter 4), where we could reduce information and selection bias, to examine OC use, parity, and breastfeeding and risk of BR-OV, OV-BR, OV, and BR using data from a study enriched for increased familial risk of breast and ovarian cancer. In the case-control study we used data from the three clinic-based sites of the Breast Cancer Family Registry (BCFR). We ran unordered polytomous logistic regression with a clustered bootstrap to adjust our standard errors given the correlated nature of our family-based data in order to examine the association between our main exposures and our four outcomes: 1) BR-OV (n = 68 cases), 2) OV-BR (n = 18 cases), 3) BR (n = 2,136 cases), and 4) OV (n = 214 cases). We observed a statistically significant inverse association between OC use and risk of BR-OV (OR=0.40, 95% CI: 0.22, 0.69) and OV-BR (OR=0.30, 95% CI: 0.05, 0.70). Similarly, we observed a statistically significant inverse association between breastfeeding and BR-OV (OR=0.49, 95% CI: 0.28, 0.84) and a suggestive non-statistically significant inverse association with OV-BR (OR=0.63, 95% CI: 0.20, 2.74). In contrast we observed a positive association between parous women with two or more children versus nulliparous and risk of BR-OV (OR=6.42, 95% CI: 2.80, 16.42) and OV-BR (OR=3.98, 95% CI: 1.01, >999). As the study cohort was enriched for increased familial risk we were able to examine whether our observed associations differed between women at high risk versus women at average risk. When we stratified by lifetime risk of breast or ovarian cancer, estimated using BOADICEA, the inverse association with OC use only remained in the average risk group while the inverse association with breastfeeding only remained in the high risk group. The positive association with parity remained in both the high and average risk groups. Overall we observed similar findings when we examined our exposures with risk of OV and BR; however, the inverse association with OC use and positive association with parity was stronger for the double primary cancer groups compared to the single primary cancer groups. As the positive association between parity and DPBOC disagreed with our original hypothesis, and the positive association with OV and BR disagree with much of the literature, we ran several sensitivity analyses to see if we could explain this association; however the positive association remained throughout. As case-control studies are subject to information and selection bias due to exposures being collected after the outcome and the challenge of identifying appropriate controls, it is possible that these

forms of bias contributed to these unexpected findings. We evaluated the impact of survivor bias by stratifying our results by pseudo-incident cases (cancers diagnosed ≤2 years prior to baseline) and prevalent cases (cancers diagnosed >2 years prior to baseline). As this type of stratification in the DPBOC group also stratifies by time between diagnosis of the first and second primary cancers, we performed an additional analysis stratifying by only one of the cancers being diagnosed ≤2 years or >2 years before baseline. While we were limited by a small sample size for the DPBOC group, these results suggested the inverse association with OC use and the positive association with parity only remained in the prevalent group. While both the inverse association with OC use and the positive association with parity were attenuated in the pseudo-incident group, the parity finding was still statistically significant. Further, the inverse association between OC use and BR and OV only remained in the prevalent group and there was no association with the pseudo-incident cases. Unequal exposure distribution between the pseudo-incident and prevalent groups and possible differential recall bias, particularly for OC use, may have contributed to this potential survivor bias. The results of this aim are summarized in Table 5.1.

Table 5.1. Summary of findings for Aim 2: Case-Control Study*

Aim 2	Main Findings			Pseudo-incident		
	DPBOC	OV	BR	DPBOC	OV	BR
Ever vs. Never OC Use	↓ BRCA -	↓ BRCA -	↓ BRCA -	ø	ø	ø
Parous vs. Nulliparous	↑	↑	↑	↑	ø	↑
Later age at first birth	↓	ø	ø	-	-	-
Later age at last birth	ø	ø	ø	-	-	-
Ever vs. Never Breastfeed	↓ø BRCA+	ø	↓	↓ø	ø	ø

* The ↑ symbol represents a positive association; the ↓ symbol represents an inverse association; the ø symbol represents a null association; the ↓ø symbol represents a non-statistically significant inverse association.

To address concerns of information and selection bias in our case-control study, we conducted a prospective cohort study to examine the association between OC use, parity, and breastfeeding and risk of BR-OV, OV, and BR (Chapter 4). Our study included data on participants from The Breast Cancer Prospective Family Study Cohort (ProF-SC) who were enrolled in the six sites of the BCFR starting in 1996 and prospectively followed (median follow-up time = 11.8 years). We used Cox proportional hazards models with a robust sandwich estimator to account for the correlated nature of our family-based data to examine the association between our main exposures and four outcomes: 1) BR-OV (n = 68 events), 2) OV-BR (n = 2 events), 3) OV (n = 68 events), and 4) BR (n = 748 events). As we only had 2 OV-BR events we were unable to examine this outcome in a multivariable model. In this study we observed a non-statistically significant positive association between OC use and risk of BR-OV (HR = 1.62, 95% CI: 0.91, 2.90) and when we stratified by BOADICEA lifetime risk of breast or ovarian cancer this result became stronger and statistically significant in women at high risk (HR = 3.05, 95% CI: 1.02, 9.13) compared to women at average risk (multiplicative interaction using continuous 1-year risk measure p = 0.06). In contrast we observed an inverse association between OC use and risk of OV. Similar to our BR-OV findings we found a positive association between OC use and risk of BR but only in older women (>52 years). Since our BR-OV group included prevalent breast cancer cases who were prospectively followed for ovarian cancer, these results may have also been influenced by survivor and recall bias. To address this concern we again stratified by time between breast cancer diagnosis and baseline interview using a two-year cutpoint. The positive association between OC use and BR-OV was only seen in the prevalent cancers and there was no association with the pseudo-incident cancers. While baseline differences between the pseudo-incident and prevalent cases may have contributed to these discordant findings, recall bias may have also played a role with differential recall of OC use. The results of this aim are summarized in Table 5.2.

Table 5.2. Summary of findings for Aim 3: Cohort Study*

Aim 3	Main Findings			Pseudo-incident
	BR-OV	OV	BR	BR-OV
Ever vs. Never OC Use	↑ High risk	↓	↑ Older women	Ø
Parous vs. Nulliparous	↓	Ø	Ø	↓ Ø
Later age at first FTP	Ø	↑	↑	-
Ever vs. Never Breastfeed	Ø	Ø	Ø	Ø

* The ↑ symbol represents a positive association; the ↓ symbol represents an inverse association; the ø symbol represents a null association; the ↓ø symbol represents a non-statistically significant inverse association.

In conclusion, when restricted to the pseudo-incident cases, the case-control and cohort studies were consistent in the finding of no association between OC use and risk of DPBOC. In contrast, the studies were contradictory in the parity and breastfeeding findings with the case-control study suggesting a positive association with parity but an inverse association with breastfeeding, and the cohort study suggesting an inverse association with parity but no association with breastfeeding. Since the pseudo-incident analysis in the cohort study was better able to mimic a cohort analysis with only the first cancer being prevalent and the second cancer being incident, and thus was able to minimize selection and information bias better than the case-control study, the findings from this analysis may be more valid.

The results from this dissertation highlight the benefits and limitations of different epidemiologic study designs in research on second primary cancers. Case-control studies, while ideal for including a sufficient number of cases, a particular concern for rare outcomes with long latency periods such as second primary cancer, are subject to information and selection bias. Recall bias, a type of information bias, is a concern in case-control studies as exposure information is collected after the outcome and reporting of exposure history may differ between cases and controls. This may be a particular problem if

having the disease results in more accurate recall of exposures or if the disease or its treatment affects memory and thus limits the accurate recall of exposures. In addition, with retrospective exposure collection in case-control studies, identifying the appropriate exposure window for a disease may be challenging. While recall of crude measures of certain exposures, such as ever versus never, may be more accurate for participants, and thus less susceptible to exposure misclassification, lack of detail on dosage or duration and timing of the exposure may result in null findings if data cannot be captured on critical periods of development when the exposure has its greatest impact on disease risk. However, in our studies we were able to observe associations with ever versus never OC use suggesting that this exposure measure was sufficient to capture an effect on disease risk. Information bias is also a concern if detailed data on these prevalent cases are unable to be obtained. While parity [172], breastfeeding [173], and OC use [174,175] have been shown to be reliably self-reported, information that requires retrieval from hospitals or registries, such as medical records, pathology reports, or tissue samples, may be more difficult to obtain for prevalent cancers, particularly if they were diagnosed long before entry into the study. Cancer confirmation is important in studies of second primary cancers in order to entirely rule out the cancer being a metastasis of the first cancer. While all of our cases were not confirmed in our case-control study, when we limited our analysis to the confirmed cases we observed minimal differences in our results. In addition, research has shown differences in histology, molecular characteristics, and survival between BR-OV and OV-BR cancers [171]; however, we were unable to examine differences by tumor histology or molecular characteristics given our small number of cases and missing pathology data. It is likely that given the rarity of second primary cancers, only large consortia will have enough power to assess risk of DPBOC by histology and molecular characteristics. While they did not assess risk of second cancer, the Ovarian Cancer Association Consortium (OCAC) examined ovarian cancer risk factors by histology and showed an inverse association between OC use and serous (the largest histologic subtype) and clear cell ovarian cancers, but no association between OC use and endometriod and mucinous ovarian cancers. While they observed an inverse association between higher parity and all histologic subtypes of ovarian cancer, it was most strongly associated with endometriod and clear cell tumors. Lastly they observed no association between duration of breastfeeding and any histologic subtype of ovarian cancer [184].

Selection bias is also a concern in case-control studies. First, the selection of an appropriate control group can be a challenge, particularly if the source population is not clearly defined. A proper control group should be a representative sample of the source population that gave rise to the cases. In our case-control study the controls were unaffected family members of the cases. While both the cases and controls came from families with breast and ovarian cancer, these families ranged in terms of both family size and number and degree of affected family members. Additionally, family members may have lived in different regions of the country which may have influenced the exposure distribution between cases and controls. Therefore, it is possible that the controls were not representative of the source population which gave rise to the cases leading to selection bias. For example, the positive association between parity and DPBOC and BR in this study could have resulted from the cases joining the study due to a concern about the health of their children which would have led to an imbalance in the distribution of the exposure, parity, between cases and controls. Second, case-control studies that use prevalent cases may be influenced by survivor bias if cases diagnosed farther from the baseline interview, and thus had longer survival before study entry, differed in the distribution of the exposure compared to cases diagnosed close to the baseline interview and thus had shorter survival before study entry. Survivor bias may have influenced the results of our case-control study as we observed differences in our OC use and parity findings between the pseudo-incident and prevalent cancers.

In the study of second primary cancers, a case-case design may limit some of the selection and information bias concerns common to case-control designs discussed above. For example, using a case-case design in our study, both the case groups (BR-OV and OV-BR) and referent groups (OV and BR, respectively) were recruited from the same source population, and were both prevalent cancers diagnosed before the baseline interview. Therefore, selection bias resulting from the control group not being representative of the source population which gave rise to the cases, as well as survivor bias, would be minimized. In addition, differential recall bias would be minimized as the exposure information was collected after a cancer diagnosis for both the case and referent groups. In Chapter 3, case-case analyses were conducted as a sensitivity analysis which assessed risk of our double primary cancer groups compared to our single primary cancer groups. While these findings were consistent with most of

our main case-control findings (an inverse association with OC use and breastfeeding, and a positive association with parity), there was no association between ever OC use and risk of BR-OV versus OV.

While cohort studies are still subject to some forms of information bias, they are less likely to be differential with respect to the exposure and outcome as exposures are collected prior to the outcome. Therefore, any misclassification is likely to be non-differential which would, in general, result in estimates being biased towards the null. However, in the study of second primary cancers where the cohort consists of women with single primary cancer being followed for the development of second primary cancer, such as is the case with our affected group (women with BR being followed for BR-OV), the exposure is collected after the first cancer diagnosis. Therefore, recall bias may be a concern, particularly with varying lengths of time between cancer diagnosis and entry into the study. In our cohort study we used crude measures of exposure (ever versus never for OC use and breastfeeding, and nulliparous versus 1 or ≥2 for parity) which would have minimized exposure misclassification but may have missed critical levels where exposure has the greatest impact on disease risk. For instance, we were unable to replicate the finding of an inverse association between parity and breast cancer risk which may have been detected if we were able to include more categories of full-term births (e.g., ≥4 full-term births). With prospective cohort studies, the ability to collect updated exposure and outcome data can reduce information bias, specifically for exposures and outcomes that can change over time, and it may be easier to examine specific exposure windows of disease risk if exposures are able to be updated prospectively. In the study of second primary cancers, exposure assessment can be a challenge as there are several times at which exposures can be assessed, such as at baseline, before the first cancer, or over time. In our cohort study we had baseline and follow-up data and were able to create time-varying covariates for our exposures that could change over time. To evaluate the influence of exposure assessment at different time points we conducted sensitivity analyses looking at just the baseline values and, for our BR-OV group, we truncated our exposures to the age of breast cancer diagnosis, rather than the age at baseline interview used in our main analyses; overall we observed minimal differences in our results.

Selection bias in cohort studies can occur with loss to follow-up. If individuals who are lost to follow-up differ in terms of the distribution of the exposure and outcome compared to individuals who

remain in the study, then the results will be biased either towards or away from the null. In the study of second primary cancers, cohort studies are also subject to survivor bias if the cohort consists of cancer survivors who are being followed prospectively for the development of a second primary cancer with varying lengths of time between diagnosis of the first cancer and the baseline interview. We observed differences in the association between OC use and risk of BR-OV when we stratified by pseudo-incident versus prevalent cancers, suggesting that survivor bias may have influenced these results. One way cohort studies examining second primary cancers can avoid this is to enroll newly-diagnosed cancers so that the baseline interview can be conducted close to the diagnosis for all participants.

Another challenge for cohort studies examining second primary cancers is to determine the appropriate follow-up start time. Follow-up start time could occur from birth, age of the first primary cancer diagnosis, or age at the baseline interview, and may be analysis-specific. For instance, if the study is designed to assess risk of second primary cancer in a cohort of women with a first primary cancer, then age at first primary cancer diagnosis may be the appropriate follow-up start time. However, if the study is designed to assess risk of a particular cancer (either first primary or second primary) in a cohort of women with our without a first primary cancer, then birth or age at interview may be the appropriate follow-up start time as everyone in the cohort will not have an age at diagnosis to start from. In our study we used different follow-up start times for our different analytic groups. For the BR-OV group we used age at breast cancer diagnosis but for the OV and BR groups we used age at baseline interview. In our sensitivity analysis where we combined the BR-OV and OV cohorts we used age at baseline interview as not everyone had an age at breast cancer diagnosis. Therefore, it is important to consider the follow-up start time when comparing results across studies.

The main challenge of cohort studies with examining second primary cancers is the ability to collect a sufficient number of cases with this outcome which has a long latency period. Therefore, the decision to design a case-control or a cohort study is often a question of precision versus validity. While case-control studies can capture more cases thus allowing for examination of a wider exposure range, and ultimately improving precision, information and selection bias may question their validity. Cohort studies, on the other hand, are less subject to information and selection bias which improves validity, but

may lack a sufficient number of cases affecting their precision, or lack detailed exposure data. However, as the exposures are collected prior to the outcome, temporality can be established and recall bias is less of a concern. While national population-based cancer registries, such as those in Denmark and Sweden, are ideal for capturing a large number of events, they often lack detailed exposure data. The ProF-SC study was uniquely designed to circumvent this lack of precision issue that cohort studies often face by enrolling breast and ovarian cancer families enriched for increased family risk. This cohort design enhances the potential for rare events, thereby increasing precision, while still capturing detailed exposure data. However, we still had an insufficient sample of OV-BR cases to assess this outcome and were likely underpowered to evaluate effect measure modification by lifetime risk.

Following the results of this dissertation, future work will focus on clarifying the discordant findings we observed between OC use, parity, and breastfeeding and risk of DPBOC in our studies by trying to replicate findings in another cohort where women are enrolled soon after their first cancer diagnosis, as well as examining the relation between other potentially modifiable risk factors and risk of DPBOC. In addition, future directions of this research will examine DNA methylation and risk of DPBOC as no studies were identified in this area. Breast and ovarian cancer have been found to have a 38% overlap in hypermethylated genes [76], showing that in addition to shared risk factors and genetics, breast and ovarian cancer also have shared epigenetics. Being able to identify epigenetic changes in the blood of women before they develop cancer could improve risk prediction models and clinical recommendations. We have already begun data collection in the BCFR to examine DNA methylation in the plasma of women affected with breast cancer who prospectively develop ovarian cancer (BR-OV) and unaffected women who prospectively develop ovarian cancer (OV).

With a growing population of cancer survivors, identifying women at greatest risk of developing a second primary cancer is critical. As breast cancer survivors are the largest group of cancer survivors in the country, understanding risk factors for the development of a second primary ovarian cancer is necessary as ovarian cancer has a high fatality rate. As there is no effective screening tool for ovarian cancer, being able to identify women at greatest risk of a second primary ovarian cancer may impact prophylactic surgery recommendations. Further, if potentially modifiable risk factors are identified, clinical

recommendations can be made for these women to improve primary prevention. As we identified a potential discordant effect of OC use on risk of second primary ovarian cancer versus first primary ovarian cancer, further work is needed to replicate this finding as OCs are commonly prescribed as a chemopreventive agent to women at high risk of ovarian cancer. Our results also suggest differences in risk factor associations by underlying risk of breast and ovarian cancer. Identifying risk factors for second primary cancer that may differ for women at high risk versus women at average risk is important for accurate clinical recommendations and personalized medicine. The results of this dissertation highlight the challenges of studying second primary cancers and methodological limitations of different study designs. Some of our results may have been influenced by survivor bias and future cohort studies should consider excluding prevalent cancers that were diagnosed more than two years prior to study entry.

References

1. SEER Stat Fact Sheets: Ovary. *http://seer.cancer.gov/statfacts/html/ovary.html* http://seer.cancer.gov/statfacts/html/ovary.html.

2. SEER Stat Fact Sheets: Female Breast. *http://seer.cancer.gov/statfacts/html/breast.html*.

3. Buys SS, Partridge E, Black A, et al. Effect of screening on ovarian cancer mortality: the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Randomized Controlled Trial. *JAMA.* 2011;305(22):2295-2303.

4. Work ME, Andrulis IL, John EM, et al. Risk factors for uncommon histologic subtypes of breast cancer using centralized pathology review in the Breast Cancer Family Registry. *Breast Cancer Res Treat.* 2012;134(3):1209-1220.

5. Work ME, John EM, Andrulis IL, et al. Reproductive risk factors and oestrogen/progesterone receptor-negative breast cancer in the Breast Cancer Family Registry. *Br J Cancer.* 2014;110(5):1367-1377.

6. Blows FM, Driver KE, Schmidt MK, et al. Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies. *PLoS Med.* 2010;7(5):e1000279.

7. Howlader N, Altekruse SF, Li CI, et al. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. *Journal of the National Cancer Institute.* 2014;106(5).

8. Kurian AW, Fish K, Shema SJ, Clarke CA. Lifetime risks of specific breast cancer subtypes among women in four racial/ethnic groups. *Breast Cancer Res.* 2010;12(6):R99.

9. Carey LA, Perou CM, Livasy CA, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA.* 2006;295(21):2492-2502.

10. Yang XR, Sherman ME, Rimm DL, et al. Differences in risk factors for breast cancer molecular subtypes in a population-based study. *Cancer Epidemiol Biomarkers Prev.* 2007;16(3):439-443.

11. Anderson WF, Chu KC, Chang S, Sherman ME. Comparison of age-specific incidence rate patterns for different histopathologic types of breast carcinoma. *Cancer Epidemiol Biomarkers Prev.* 2004;13(7):1128-1135.

12. Li CI, Uribe DJ, Daling JR. Clinical characteristics of different histologic types of breast cancer. *Br J Cancer.* 2005;93(9):1046-1052.

13. Reeves GK, Beral V, Green J, Gathani T, Bull D, Million Women Study C. Hormonal therapy for menopause and breast-cancer risk by histological type: a cohort study and meta-analysis. *Lancet Oncol.* 2006;7(11):910-918.

14. Chen VW, Ruiz B, Killeen JL, Cote TR, Wu XC, Correa CN. Pathology and classification of ovarian tumors. *Cancer.* 2003;97(10 Suppl):2631-2642.

15. Toss A, Tomasello C, Razzaboni E, et al. Hereditary ovarian cancer: not only BRCA 1 and 2 genes. *Biomed Res Int.* 2015;2015:341723.

16. Kurman RJ, Shih Ie M. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer--shifting the paradigm. *Hum Pathol.* 2011;42(7):918-931.

17. McCluggage WG. Morphological subtypes of ovarian carcinoma: a review with emphasis on new developments and pathogenesis. *Pathology.* 2011;43(5):420-432.

18. Kobel M, Kalloger SE, Huntsman DG, et al. Differences in tumor type in low-stage versus high-stage ovarian carcinomas. *Int J Gynecol Pathol.* 2010;29(3):203-211.

19. Seidman JD, Horkayne-Szakaly I, Haiba M, Boice CR, Kurman RJ, Ronnett BM. The histologic type and stage distribution of ovarian carcinomas of surface epithelial origin. *Int J Gynecol Pathol.* 2004;23(1):41-44.

20. Yang HP, Anderson WF, Rosenberg PS, et al. Ovarian cancer incidence trends in relation to changing patterns of menopausal hormone therapy use in the United States. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2013;31(17):2146-2151.

21. *NCCN Clinical Guidelines in Oncology. Genetic/Familial High-Risk Assessment: Breast and Ovarian.* Version 1.2018.

22. Boyd NF, Martin LJ, Rommens JM, et al. Mammographic density: a heritable risk factor for breast cancer. *Methods Mol Biol.* 2009;472:343-360.

23. Russo A, Calo V, Bruno L, Rizzo S, Bazan V, Di Fede G. Hereditary ovarian cancer. *Crit Rev Oncol Hematol.* 2009;69(1):28-44.

24. Metcalfe KA, Finch A, Poll A, et al. Breast cancer risks in women with a family history of breast or ovarian cancer who have tested negative for a BRCA1 or BRCA2 mutation. *Br J Cancer.* 2009;100(2):421-425.

25. Shih HA, Couch FJ, Nathanson KL, et al. BRCA1 and BRCA2 mutation frequency in women evaluated in a breast cancer risk evaluation clinic. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2002;20(4):994-999.

26. Simard J, Dumont M, Moisan AM, et al. Evaluation of BRCA1 and BRCA2 mutation prevalence, risk prediction models and a multistep testing approach in French-Canadian families with high risk of breast and ovarian cancer. *Journal of medical genetics.* 2007;44(2):107-121.

27. Ramus SJ, Harrington PA, Pye C, et al. Contribution of BRCA1 and BRCA2 mutations to inherited ovarian cancer. *Hum Mutat.* 2007;28(12):1207-1215.

28. Kauff ND, Mitra N, Robson ME, et al. Risk of ovarian cancer in BRCA1 and BRCA2 mutation-negative hereditary breast cancer families. *Journal of the National Cancer Institute.* 2005;97(18):1382-1384.

29. Yoon PW, Scheuner MT, Peterson-Oehlke KL, Gwinn M, Faucett A, Khoury MJ. Can family history be used as a tool for public health and preventive medicine? *Genetics in medicine : official journal of the American College of Medical Genetics.* 2002;4(4):304-310.

30. Stratton JF, Pharoah P, Smith SK, Easton D, Ponder BA. A systematic review and meta-analysis of family history and risk of ovarian cancer. *Br J Obstet Gynaecol.* 1998;105(5):493-499.

31. Nelson HD, Cantor A, Humphrey L, et al. *Screening for Breast Cancer: A Systematic Review to Update the 2009 U.S. Preventive Services Task Force Recommendation*. Rockville (MD)2016.

32. Jacobs IJ, Menon U, Ryan A, et al. Ovarian cancer screening and mortality in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial. *Lancet.* 2016;387(10022):945-956.

33. Pinsky PF, Zhu C, Skates SJ, et al. Potential effect of the risk of ovarian cancer algorithm (ROCA) on the mortality outcome of the Prostate, Lung, Colorectal and Ovarian (PLCO) trial. *Int J Cancer.* 2013;132(9):2127-2133.

34. Bergfeldt K, Rydh B, Granath F, et al. Risk of ovarian cancer in breast-cancer patients with a family history of breast or ovarian cancer: a population-based cohort study. *Lancet.* 2002;360(9337):891-894.

35. Berkowitz Z, Rim SH, Peipins LA. Characteristics and survival associated with ovarian cancer diagnosed as first cancer and ovarian cancer diagnosed subsequent to a previous cancer. *Cancer epidemiology.* 2011;35(2):112-119.

36. Hall HI, Jamison P, Weir HK. Second primary ovarian cancer among women diagnosed previously with cancer. *Cancer Epidemiol Biomarkers Prev.* 2001;10(9):995-999.

37. Harvey EB, Brinton LA. Second cancer following cancer of the breast in Connecticut, 1935-82. *National Cancer Institute monograph.* 1985;68:99-112.

38. Schonfeld SJ, Berrington de Gonzalez A, Visvanathan K, Pfeiffer RM, Anderson WF. Declining second primary ovarian cancer after first primary breast cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2013;31(6):738-743.

39. Soerjomataram I, Louwman WJ, Lemmens VE, de Vries E, Klokman WJ, Coebergh JW. Risks of second primary breast and urogenital cancer following female breast cancer in the south of The Netherlands, 1972-2001. *Eur J Cancer.* 2005;41(15):2331-2337.

40. Travis LB, Curtis RE, Boice JD, Jr., Platz CE, Hankey BF, Fraumeni JF, Jr. Second malignant neoplasms among long-term survivors of ovarian cancer. *Cancer research.* 1996;56(7):1564-1570.

41. van Niekerk CC, Vooijs GP, Bulten J, van Dijck JA, Verbeek AL. Increased risk of concurrent primary malignancies in patients diagnosed with a primary malignant epithelial ovarian tumor. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc.* 2007;20(3):384-388.

42. Curtis RE, Ron E, Hankey BF, Hoover RN. New Malignancies Following Breast Cancer. In: Curtis RE, Freedman DM, Ron E, Ries LAG, Hacker DG, Edwards BK, Tucker MA, Fraumeni JF Jr, ed. *New Malignancies Among Cancer Survivors: SEER Cancer Registries, 1973-2000*: National Cancer Institute, NIH Publ. No. 05-5302. Bethesda, MD; 2006:181-205.

43. Kirova YM, De Rycke Y, Gambotti L, et al. Second malignancies after breast cancer: the impact of different treatment modalities. *Br J Cancer.* 2008;98(5):870-874.

44. Freedman DM, Curtis RE, Travis LB, Fraumeni JF Jr. New Malignancies Following Cancer of the Uterine Corpus and Ovary. In: Curtis RE, Freedman DM, Ron E, Ries LAG, Hacker DG, Edwards BK, Tucker MA, Fraumeni JF Jr, ed. *New Malignancies Among Cancer Survivors: SEER Cancer Registries, 1973-2000*: Nantional Cancer Institute, NIH Publ. No. 05-5302; Bethesda, MD, 2006.

45. Zografos GC, Panou M, Panou N. Common risk factors of breast and ovarian cancer: recent view. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society.* 2004;14(5):721-740.

46. Permuth-Wey J, Sellers TA. Epidemiology of ovarian cancer. *Methods Mol Biol.* 2009;472:413-437.

47. Cibula D, Zikan M, Dusek L, Majek O. Oral contraceptives and risk of ovarian and breast cancers in BRCA mutation carriers: a meta-analysis. *Expert Rev Anticancer Ther.* 2011;11(8):1197-1207.

48. Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. Collaborative Group on Hormonal Factors in Breast Cancer. *Lancet.* 1996;347(9017):1713-1727.

49. Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. *Epidemiologic reviews.* 1993;15(1):36-47.

50. Narod SA. Modifiers of risk of hereditary breast cancer. *Oncogene.* 2006;25(43):5832-5836.

51. Albrektsen G, Heuch I, Kvale G. Reproductive factors and incidence of epithelial ovarian cancer: a Norwegian prospective study. *Cancer Causes Control.* 1996;7(4):421-427.

52. Cooper GS, Schildkraut JM, Whittemore AS, Marchbanks PA. Pregnancy recency and risk of ovarian cancer. *Cancer Causes Control.* 1999;10(5):397-402.

53. Titus-Ernstoff L, Perez K, Cramer DW, Harlow BL, Baron JA, Greenberg ER. Menstrual and reproductive factors in relation to ovarian cancer risk. *Br J Cancer.* 2001;84(5):714-721.

54. Whiteman DC, Siskind V, Purdie DM, Green AC. Timing of pregnancy and the risk of epithelial ovarian cancer. *Cancer Epidemiol Biomarkers Prev.* 2003;12(1):42-46.

55. Gronwald J, Byrski T, Huzarski T, et al. Influence of selected lifestyle factors on breast and ovarian cancer risk in BRCA1 mutation carriers from Poland. *Breast Cancer Res Treat.* 2006;95(2):105-109.

56. Antoniou AC, Rookus M, Andrieu N, et al. Reproductive and hormonal factors, and ovarian cancer risk for BRCA1 and BRCA2 mutation carriers: results from the International BRCA1/2 Carrier Cohort Study. *Cancer Epidemiol Biomarkers Prev.* 2009;18(2):601-610.

57. McLaughlin JR, Risch HA, Lubinski J, et al. Reproductive risk factors for ovarian cancer in carriers of BRCA1 or BRCA2 mutations: a case-control study. *Lancet Oncol.* 2007;8(1):26-34.

58. Ferris JS, Daly MB, Buys SS, Genkinger JM, Liao Y, Terry MB. Oral contraceptive and reproductive risk factors for ovarian cancer within sisters in the breast cancer family registry. *Br J Cancer.* 2014;110(4):1074-1080.

59. McGuire V, Felberg A, Mills M, et al. Relation of contraceptive and reproductive history to ovarian cancer risk in carriers and noncarriers of BRCA1 gene mutations. *Am J Epidemiol.* 2004;160(7):613-618.

60. Modan B, Hartge P, Hirsh-Yechezkel G, et al. Parity, oral contraceptives, and the risk of ovarian cancer among carriers and noncarriers of a BRCA1 or BRCA2 mutation. *N Engl J Med.* 2001;345(4):235-240.

61. Kotsopoulos J, Lubinski J, Lynch HT, et al. Age at first birth and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. *Breast Cancer Res Treat.* 2007;105(2):221-228.

62. Milne RL, Osorio A, Ramon y Cajal T, et al. Parity and the risk of breast and ovarian cancer in BRCA1 and BRCA2 mutation carriers. *Breast Cancer Res Treat.* 2010;119(1):221-232.

63. Rebbeck TR, Wang Y, Kantoff PW, et al. Modification of BRCA1- and BRCA2-associated breast cancer risk by AIB1 genotype and reproductive history. *Cancer research.* 2001;61(14):5420-5424.

64. Antoniou AC, Shenton A, Maher ER, et al. Parity and breast cancer risk among BRCA1 and BRCA2 mutation carriers. *Breast Cancer Res.* 2006;8(6):R72.

65. Chang-Claude J, Andrieu N, Rookus M, et al. Age at menarche and menopause and breast cancer risk in the International BRCA1/2 Carrier Cohort Study. *Cancer Epidemiol Biomarkers Prev.* 2007;16(4):740-746.

66. Kotsopoulos J, Lubinski J, Gronwald J, et al. Factors influencing ovulation and the risk of ovarian cancer in BRCA1 and BRCA2 mutation carriers. *Int J Cancer.* 2015;137(5):1136-1146.

67. Kotsopoulos J, Huzarski T, Gronwald J, et al. Hormone replacement therapy after menopause and risk of breast cancer in BRCA1 mutation carriers: a case-control study. *Breast Cancer Res Treat.* 2016;155(2):365-373.

68. Kotsopoulos J, Lubinski J, Neuhausen SL, et al. Hormone replacement therapy and the risk of ovarian cancer in BRCA1 and BRCA2 mutation carriers. *Gynecologic oncology.* 2006;100(1):83-88.

69. Njiaju UO, Olopade OI. Genetic determinants of breast cancer risk: a review of current literature and issues pertaining to clinical application. *Breast J.* 2012;18(5):436-442.

70. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. *Nature.* 2012;490(7418):61-70.

71. Cancer Genome Atlas Research N. Integrated genomic analyses of ovarian carcinoma. *Nature.* 2011;474(7353):609-615.

72. Petrucelli N, Daly MB, Feldman GL. Hereditary breast and ovarian cancer due to mutations in BRCA1 and BRCA2. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2010;12(5):245-259.

73. Feinberg AP. The epigenetics of cancer etiology. *Seminars in cancer biology.* 2004;14(6):427-432.

74. Jones PA. DNA methylation and cancer. *Oncogene.* 2002;21(35):5358-5360.

75. Terry MB, Ferris JS, Pilsner R, et al. Genomic DNA methylation among women in a multiethnic New York City birth cohort. *Cancer Epidemiol Biomarkers Prev.* 2008;17(9):2306-2310.

76. Heichman KA, Warren JD. DNA methylation biomarkers and their utility for solid cancer diagnostics. *Clinical chemistry and laboratory medicine : CCLM / FESCC.* 2012;50(10):1707-1721.

77. Centers for Disease Control and Prevention: Cancer Survivorship. *https://www.cdc.gov/cancer/survivorship/basic_info/survivors/index.htm*. 2017.

78. Hayat MJ, Howlader N, Reichman ME, Edwards BK. Cancer statistics, trends, and multiple primary cancer analyses from the Surveillance, Epidemiology, and End Results (SEER) Program. *The oncologist.* 2007;12(1):20-37.

79. Travis LB. Therapy-associated solid tumors. *Acta Oncol.* 2002;41(4):323-333.

80. Wood ME, Vogel V, Ng A, Foxhall L, Goodwin P, Travis LB. Second malignant neoplasms: assessment and strategies for risk reduction. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2012;30(30):3734-3745.

81. Travis LB, Rabkin CS, Brown LM, et al. Cancer survivorship--genetic susceptibility and second primary cancers: research strategies and recommendations. *Journal of the National Cancer Institute.* 2006;98(1):15-25.

82. Van Leeuwen FEaT, L. B. Second Cancers. In: Devita VT, Hellman, S., Rosenberg, S. A., ed. *Cancer: Principles and Practice of Oncology*. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2005:2575-2602.

83. Garber JE, Offit K. Hereditary cancer predisposition syndromes. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2005;23(2):276-292.

84. Begg CB, Zhang ZF, Sun M, Herr HW, Schantz SP. Methodology for evaluating the incidence of second primary cancers with application to smoking-related cancers from the Surveillance, Epidemiology, and End Results (SEER) program. *Am J Epidemiol.* 1995;142(6):653-665.

85. *American Cancer Society. Cancer Facts & Figures 2013.* Atlanta, GA: American Cancer Society;2013.

86. Trentham-Dietz A, Newcomb PA, Nichols HB, Hampton JM. Breast cancer risk factors and second primary malignancies among women with breast cancer. *Breast Cancer Res Treat.* 2007;105(2):195-207.

87. Bergfeldt K, Nilsson B, Einhorn S, Hall P. Breast cancer risk in women with a primary ovarian cancer--a case-control study. *Eur J Cancer.* 2001;37(17):2229-2234.

88. Cvelbar M, Ursic-Vrscaj M, Rakar S. Risk factors and prognostic factors in patients with double primary cancer: epithelial ovarian cancer and breast cancer. *European journal of gynaecological oncology.* 2005;26(1):59-63.

89. Langballe R, Olsen JH, Andersson M, Mellemkjaer L. Risk for second primary non-breast cancer in pre- and postmenopausal women with breast cancer not treated with chemotherapy, radiotherapy or endocrine therapy. *Eur J Cancer.* 2011;47(6):946-952.

90. Metcalfe KA, Lynch HT, Ghadirian P, et al. The risk of ovarian cancer after breast cancer in BRCA1 and BRCA2 carriers. *Gynecologic oncology.* 2005;96(1):222-226.

91. Iwasa Z, Jinnai D, Koyama H, Sasano N. Second primary cancer following adjuvant chemotherapy, radiotherapy and endocrine therapy for breast cancer: a nationwide survey on 47,005 Japanese patients who underwent mastectomy from 1963-1982. *Jpn J Surg.* 1986;16(4):262-271.

92. Andersson M, Jensen MB, Engholm G, Henrik Storm H. Risk of second primary cancer among patients with early operable breast cancer registered or randomised in Danish Breast Cancer cooperative Group (DBCG) protocols of the 77, 82 and 89 programmes during 1977-2001. *Acta Oncol.* 2008;47(4):755-764.

93. Tanaka H, Tsukuma H, Koyama H, Kinoshita Y, Kinoshita N, Oshima A. Second primary cancers following breast cancer in the Japanese female population. *Jpn J Cancer Res.* 2001;92(1):1-8.

94. Andersson M, Storm HH, Mouridsen HT. Incidence of new primary cancers after adjuvant tamoxifen therapy and radiotherapy for early breast cancer. *Journal of the National Cancer Institute.* 1991;83(14):1013-1017.

95. Grantzau T, Mellemkjaer L, Overgaard J. Second primary cancers after adjuvant radiotherapy in early breast cancer patients: a national population based study under the Danish Breast Cancer Cooperative Group (DBCG). *Radiother Oncol.* 2013;106(1):42-49.

96. Rubino C, de Vathaire F, Diallo I, Shamsaldin A, Le MG. Increased risk of second cancers following breast cancer: role of the initial treatment. *Breast Cancer Res Treat.* 2000;61(3):183-195.

97. Berrington de Gonzalez A, Curtis RE, Gilbert E, et al. Second solid cancers after radiotherapy for breast cancer in SEER cancer registries. *Br J Cancer.* 2010;102(1):220-226.

98. Reimer RR, Hoover R, Fraumeni JF, Jr., Young RC. Second primary neoplasms following ovarian cancer. *Journal of the National Cancer Institute.* 1978;61(5):1195-1197.

99. Cook LS, Weiss NS, Schwartz SM, et al. Population-based study of tamoxifen therapy and subsequent ovarian, endometrial, and breast cancers. *Journal of the National Cancer Institute.* 1995;87(18):1359-1364.

100. Curtis RE, Boice JD, Jr., Shriner DA, Hankey BF, Fraumeni JF, Jr. Second cancers after adjuvant tamoxifen therapy for breast cancer. *Journal of the National Cancer Institute.* 1996;88(12):832-834.

101. Yadav BS, Sharma SC, Patel FD, Ghoshal S, Kapoor R, Kumar R. Nonbreast second malignancies after treatment of primary breast cancer. *Int J Radiat Oncol Biol Phys.* 2009;73(5):1489-1492.

102. Fishman A, Dekel E, Chetrit A, et al. Patients with double primary tumors in the breast and ovary-clinical characteristics and BRCA1-2 mutations status. *Gynecologic oncology.* 2000;79(1):74-78.

103. Schorge JO, Mahoney NM, Miller DS, et al. Germline BRCA1-2 mutations in non-Ashkenazi families with double primary breast and ovarian cancer. *Gynecologic oncology.* 2001;83(2):383-387.

104. Einbeigi Z, Bergman A, Meis-Kindblom JM, et al. Occurrence of both breast and ovarian cancer in a woman is a marker for the BRCA gene mutations: a population-based study from western Sweden. *Fam Cancer.* 2007;6(1):35-41.

105. Cvelbar M, Hocevar M, Vidmar G, Teugels E. BRCA1/2 status and clinicopathologic characteristics of patients with double primary breast and ovarian cancer. *Neoplasma.* 2011;58(3):198-204.

106. Bruchim I, Fishman A, Friedman E, et al. Analyses of p53 expression pattern and BRCA mutations in patients with double primary breast and ovarian cancer. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society.* 2004;14(2):251-258.

107. Pilarski R, Patel DA, Weitzel J, et al. The KRAS-variant is associated with risk of developing double primary breast and ovarian cancer. *PloS one.* 2012;7(5):e37891.

108. Evans DG, Ahmed M, Bayliss S, Howard E, Lalloo F, Wallace A. BRCA1, BRCA2 and CHEK2 c.1100 delC mutations in patients with double primaries of the breasts and/or ovaries. *Journal of medical genetics.* 2010;47(8):561-566.

109. Domchek SM, Jhaveri K, Patil S, et al. Risk of metachronous breast cancer after BRCA mutation-associated ovarian cancer. *Cancer.* 2013;119(7):1344-1348.

110. Gangi A, Cass I, Paik D, et al. Breast cancer following ovarian cancer in BRCA mutation carriers. *JAMA Surg.* 2014;149(12):1306-1313.

111. Vencken PM, Kriege M, Hooning M, et al. The risk of primary and contralateral breast cancer after ovarian cancer in BRCA1/BRCA2 mutation carriers: Implications for counseling. *Cancer.* 2013;119(5):955-962.

112. Prochazka M, Hall P, Granath F, Czene K. Family history of breast cancer and young age at diagnosis of breast cancer increase risk of second primary malignancies in women: a population-based cohort study. *Br J Cancer.* 2006;95(9):1291-1295.

113. Hemminki K, Zhang H, Sundquist J, Lorenzo Bermejo J. Modification of risk for subsequent cancer after female breast cancer by a family history of breast cancer. *Breast Cancer Res Treat.* 2008;111(1):165-169.

114. Lee E, Ma H, McKean-Cowdin R, et al. Effect of reproductive factors and oral contraceptives on breast cancer risk in BRCA1/2 mutation carriers and noncarriers: results from a population-based study. *Cancer Epidemiol Biomarkers Prev.* 2008;17(11):3170-3178.

115. Cullinane CA, Lubinski J, Neuhausen SL, et al. Effect of pregnancy as a risk factor for breast cancer in BRCA1/BRCA2 mutation carriers. *Int J Cancer.* 2005;117(6):988-991.

116. Kahlenborn C, Modugno F, Potter DM, Severs WB. Oral contraceptive use as a risk factor for premenopausal breast cancer: a meta-analysis. *Mayo Clin Proc.* 2006;81(10):1290-1302.

117. Kumle M, Weiderpass E, Braaten T, Persson I, Adami HO, Lund E. Use of oral contraceptives and breast cancer risk: The Norwegian-Swedish Women's Lifestyle and Health Cohort Study. *Cancer Epidemiol Biomarkers Prev.* 2002;11(11):1375-1381.

118. Hankinson SE, Colditz GA, Manson JE, et al. A prospective study of oral contraceptive use and risk of breast cancer (Nurses' Health Study, United States). *Cancer Causes Control.* 1997;8(1):65-72.

119. Hannaford PC, Selvaraj S, Elliott AM, Angus V, Iversen L, Lee AJ. Cancer risk among users of oral contraceptives: cohort data from the Royal College of General Practitioner's oral contraception study. *BMJ.* 2007;335(7621):651.

120. Marchbanks PA, McDonald JA, Wilson HG, et al. Oral contraceptives and the risk of breast cancer. *N Engl J Med.* 2002;346(26):2025-2032.

121. Vessey M, Painter R. Oral contraceptive use and cancer. Findings in a large cohort study, 1968-2004. *Br J Cancer.* 2006;95(3):385-389.

122. Fishman A. The effects of parity, breastfeeding, and infertility treatment on the risk of hereditary breast and ovarian cancer: a review. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society.* 2010;20(11 Suppl 2):S31-33.

123. Jernstrom H, Lubinski J, Lynch HT, et al. Breast-feeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. *Journal of the National Cancer Institute.* 2004;96(14):1094-1098.

124. Kotsopoulos J, Lubinski J, Salmena L, et al. Breastfeeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. *Breast Cancer Res.* 2012;14(2):R42.

125. Delgado-Cruzata L, Wu HC, Perrin M, et al. Global DNA methylation levels in white blood cell DNA from sisters discordant for breast cancer from the New York site of the Breast Cancer Family Registry. *Epigenetics : official journal of the DNA Methylation Society.* 2012;7(8):868-874.

126. John EM, Hopper JL, Beck JC, et al. The Breast Cancer Family Registry: an infrastructure for cooperative multinational, interdisciplinary and translational studies of the genetic epidemiology of breast cancer. *Breast Cancer Res.* 2004;6(4):R375-389.

127. Kennedy DO, Agrawal M, Shen J, et al. DNA repair capacity of lymphoblastoid cell lines from sisters discordant for breast cancer. *Journal of the National Cancer Institute.* 2005;97(2):127-132.

128. Machella N, Terry MB, Zipprich J, et al. Double-strand breaks repair in lymphoblastoid cell lines from sisters discordant for breast cancer from the New York site of the BCFR. *Carcinogenesis.* 2008;29(7):1367-1372.

129. Shen J, Terry MB, Gurvich I, Liao Y, Senie RT, Santella RM. Short telomere length and breast cancer risk: a study in sister sets. *Cancer research.* 2007;67(11):5538-5544.

130. Shen J, Terry MB, Liao Y, et al. Genetic variation in telomere maintenance genes, telomere length and breast cancer risk. *PloS one.* 2012;7(9):e44308.

131. Terry MB, Knight JA, Zablotska L, et al. Alcohol metabolism, alcohol intake, and breast cancer risk: a sister-set analysis using the Breast Cancer Family Registry. *Breast Cancer Res Treat.* 2007;106(2):281-288.

132. Wu HC, Delgado-Cruzata L, Flom JD, et al. Global methylation profiles in DNA from different blood cell types. *Epigenetics : official journal of the DNA Methylation Society.* 2011;6(1):76-85.

133. Wu HC, Delgado-Cruzata L, Flom JD, et al. Repetitive element DNA methylation levels in white blood cell DNA from sisters discordant for breast cancer from the New York site of the Breast Cancer Family Registry. *Carcinogenesis.* 2012;33(10):1946-1952.

134. Yazici H, Terry MB, Cho YH, et al. Aberrant methylation of RASSF1A in plasma DNA before breast cancer diagnosis in the Breast Cancer Family Registry. *Cancer Epidemiol Biomarkers Prev.* 2009;18(10):2723-2725.

135. Zipprich J, Terry MB, Liao Y, et al. Plasma protein carbonyls and breast cancer risk in sisters discordant for breast cancer from the New York site of the Breast Cancer Family Registry. *Cancer research.* 2009;69(7):2966-2972.

136. Neuhausen SL, Ozcelik H, Southey MC, et al. BRCA1 and BRCA2 mutation carriers in the Breast Cancer Family Registry: an open resource for collaborative research. *Breast Cancer Res Treat.* 2009;116(2):379-386.

137. de Rooij M, Worku HM. A warning concerning the estimation of multinomial logistic models with correlated responses in SAS. *Computer methods and programs in biomedicine.* 2012;107(2):341-346.

138. Lee AJ, Cunningham AP, Kuchenbaecker KB, et al. BOADICEA breast cancer risk prediction model: updates to cancer incidences, tumour pathology and web interface. *Br J Cancer.* 2014;110(2):535-545.

139. Bevers TB, Anderson BO, Bonaccio E, et al. NCCN clinical practice guidelines in oncology: breast cancer screening and diagnosis. *J Natl Compr Canc Netw.* 2009;7(10):1060-1096.

140. Shantakumar S, Terry MB, Paykin A, et al. Age and menopausal effects of hormonal birth control and hormone replacement therapy in relation to breast cancer risk. *Am J Epidemiol.* 2007;165(10):1187-1198.

141. Andersson T, Alfredsson L, Kallberg H, Zdravkovic S, Ahlbom A. Calculating measures of biological interaction. *Eur J Epidemiol.* 2005;20(7):575-579.

142. Collaborative Group on Epidemiological Studies of Ovarian C, Beral V, Doll R, Hermon C, Peto R, Reeves G. Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23,257 women with ovarian cancer and 87,303 controls. *Lancet.* 2008;371(9609):303-314.

143. Iodice S, Barile M, Rotmensz N, et al. Oral contraceptive use and breast or ovarian cancer risk in BRCA1/2 carriers: a meta-analysis. *Eur J Cancer.* 2010;46(12):2275-2284.

144. Moorman PG, Havrilesky LJ, Gierisch JM, et al. Oral contraceptives and risk of ovarian cancer and breast cancer among high-risk women: a systematic review and meta-analysis. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2013;31(33):4188-4198.

145. Collaborative Group on Hormonal Factors in Breast C. Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. *Lancet.* 1996;347(9017):1713-1727.

146. Milne RL, Knight JA, John EM, et al. Oral contraceptive use and risk of early-onset breast cancer in carriers and noncarriers of BRCA1 and BRCA2 mutations. *Cancer Epidemiol Biomarkers Prev.* 2005;14(2):350-356.

147. Collaborative Group on Hormonal Factors in Breast C. Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. *Lancet.* 2001;358(9291):1389-1399.

148. Lambe M, Hsieh C, Trichopoulos D, Ekbom A, Pavia M, Adami HO. Transient increase in the risk of breast cancer after giving birth. *N Engl J Med.* 1994;331(1):5-9.

149. Albrektsen G, Heuch I, Hansen S, Kvale G. Breast cancer risk by age at birth, time since birth and time intervals between births: exploring interaction effects. *Br J Cancer.* 2005;92(1):167-175.

150. Gnagy S, Ming EE, Devesa SS, Hartge P, Whittemore AS. Declining ovarian cancer rates in U.S. women in relation to parity and oral contraceptive use. *Epidemiology.* 2000;11(2):102-105.

151. Russo J, Lynch H, Russo IH. Mammary gland architecture as a determining factor in the susceptibility of the human breast to cancer. *Breast J.* 2001;7(5):278-291.

152. Collaborative Group on Hormonal Factors in Breast C. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. *Lancet.* 2002;360(9328):187-195.

153. Booth M, Beral V, Smith P. Risk factors for ovarian cancer: a case-control study. *Br J Cancer.* 1989;60(4):592-598.

154. Franceschi S, La Vecchia C, Helmrich SP, Mangioni C, Tognoni G. Risk factors for epithelial ovarian cancer in Italy. *Am J Epidemiol.* 1982;115(5):714-719.

155. Greggi S, Parazzini F, Paratore MP, et al. Risk factors for ovarian cancer in central Italy. *Gynecologic oncology.* 2000;79(1):50-54.

156. Hildreth NG, Kelsey JL, LiVolsi VA, et al. An epidemiologic study of epithelial carcinoma of the ovary. *Am J Epidemiol.* 1981;114(3):398-405.

157. Horn-Ross PL, Whittemore AS, Harris R, Itnyre J. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 U.S. case-control studies. VI. Nonepithelial cancers among adults. Collaborative Ovarian Cancer Group. *Epidemiology.* 1992;3(6):490-495.

158. Joly DJ, Lilienfeld AM, Diamond EL, Bross ID. An epidemiologic study of the relationship of reproductive experience to cancer of the ovary. *Am J Epidemiol.* 1974;99(3):190-209.

159. La Vecchia C, Decarli A, Franceschi S, Regallo M, Tognoni G. Age at first birth and the risk of epithelial ovarian cancer. *Journal of the National Cancer Institute.* 1984;73(3):663-666.

160. McGowan L, Norris HJ, Hartge P, Hoover R, Lesher L. Risk factors in ovarian cancer. *European journal of gynaecological oncology.* 1988;9(3):195-199.

161. Negri E, Franceschi S, Tzonou A, et al. Pooled analysis of 3 European case-control studies: I. Reproductive factors and risk of epithelial ovarian cancer. *Int J Cancer.* 1991;49(1):50-56.

162. Tavani A, Negri E, Franceschi S, Parazzini F, La Vecchia C. Risk factors for epithelial ovarian cancer in women under age 45. *Eur J Cancer.* 1993;29A(9):1297-1301.

163. Purdie D, Green A, Bain C, et al. Reproductive and other factors and risk of epithelial ovarian cancer: an Australian case-control study. Survey of Women's Health Study Group. *Int J Cancer.* 1995;62(6):678-684.

164. Whittemore AS, Harris R, Itnyre J. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. II. Invasive epithelial ovarian cancers in white women. Collaborative Ovarian Cancer Group. *Am J Epidemiol.* 1992;136(10):1184-1203.

165. Adami HO, Hsieh CC, Lambe M, et al. Parity, age at first childbirth, and risk of ovarian cancer. *Lancet.* 1994;344(8932):1250-1254.

166. Hankinson SE, Colditz GA, Hunter DJ, et al. A prospective study of reproductive factors and risk of epithelial ovarian cancer. *Cancer.* 1995;76(2):284-290.

167. Hajdu SI, Urban JA. Cancers metastatic to the breast. *Cancer.* 1972;29(6):1691-1696.

168. Laifer S, Buscema J, Parmley TH, Rosenshein NB. Ovarian cancer metastatic to the breast. *Gynecologic oncology.* 1986;24(1):97-102.

169. Curtin JP, Barakat RR, Hoskins WJ. Ovarian disease in women with breast cancer. *Obstetrics and gynecology.* 1994;84(3):449-452.

170. Young RH. From Krukenberg to today: the ever present problems posed by metastatic tumors in the ovary. Part II. *Adv Anat Pathol.* 2007;14(3):149-177.

171. Liou WS, Hamilton CA, Cheung MK, et al. Outcomes of women with metachronous breast and ovarian carcinomas. *Gynecologic oncology.* 2006;103(1):190-194.

172. Harlow SD, Linet MS. Agreement between questionnaire data and medical records. The evidence for accuracy of recall. *Am J Epidemiol.* 1989;129(2):233-248.

173. Li R, Scanlon KS, Serdula MK. The validity and reliability of maternal recall of breastfeeding practice. *Nutr Rev.* 2005;63(4):103-110.

174. Oakley D, Sereika S, Bogue EL. Oral contraceptive pill use after an initial visit to a family planning clinic. *Family planning perspectives.* 1991;23(4):150-154.

175. Spangler L, Grafton J, Ichikawa L, et al. PS2-29: Comparison of Self-reported Oral Contraceptive Use and Automated Prescription Fills. *Clinical medicine & research.* 2012;10(3):186-187.

176. *American Cancer Socity. Cancer Treatment & Survivorship Facts & Figures 2016-2017.* Atlanta: American Cancer Socity; 2016.

177. Curtis RE, Freedman DM, Ron E, et al. *New Malignancies Among Cancer Survivors: SEER Cancer Registries, 1973-2000.* National Cancer Institute,NIH Publ. No. 05-5302. Bethesda, MD, 2006.

178. Escobar PF, Patrick R, Rybicki L, Al-Husaini N, Michener CM, Crowe JP. Primary gynecological neoplasms and clinical outcomes in patients diagnosed with breast carcinoma. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society.* 2006;16 Suppl 1:118-122.

179. Raymond JS, Hogue CJ. Multiple primary tumours in women following breast cancer, 1973-2000. *Br J Cancer.* 2006;94(11):1745-1750.

180. Ji J, Forsti A, Sundquist J, Lenner P, Hemminki K. Survival in ovarian cancer patients by histology and family history. *Acta Oncol.* 2008;47(6):1133-1139.

181. Terry MB, Phillips KA, Daly MB, et al. Cohort Profile: The Breast Cancer Prospective Family Study Cohort (ProF-SC). *International journal of epidemiology.* 2016;45(3):683-692.

182. Lin DY, Wei LJ, Ying Z. Checking the Cox Model with Cumulative Sums of Martingale-Based Residuals. *Biometrika.* 1993;80(3):557-572.

183. Yang HP, Trabert B, Murphy MA, et al. Ovarian cancer risk factors by histologic subtypes in the NIH-AARP Diet and Health Study. *Int J Cancer.* 2012;131(4):938-948.

184. Wentzensen N, Poole EM, Trabert B, et al. Ovarian Cancer Risk Factors by Histologic Subtype: An Analysis From the Ovarian Cancer Cohort Consortium. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2016;34(24):2888-2898.

185. Rookus MA, van Leeuwen FE. Oral contraceptives and risk of breast cancer in women aged 20-54 years. Netherlands Oral Contraceptives and Breast Cancer Study Group. *Lancet.* 1994;344(8926):844-851.

186. Wingo PA, Lee NC, Ory HW, Beral V, Peterson HB, Rhodes P. Age-specific differences in the relationship between oral contraceptive use and breast cancer. *Cancer.* 1993;71(4 Suppl):1506-1517.

187. Feng LP, Chen HL, Shen MY. Breastfeeding and the risk of ovarian cancer: a meta-analysis. *J Midwifery Womens Health.* 2014;59(4):428-437.

188. Li DP, Du C, Zhang ZM, et al. Breastfeeding and ovarian cancer risk: a systematic review and meta-analysis of 40 epidemiological studies. *Asian Pac J Cancer Prev.* 2014;15(12):4829-4837.

189. Becher H, Schmidt S, Chang-Claude J. Reproductive factors and familial predisposition for breast cancer by age 50 years. A case-control-family study for assessing main effects and possible gene-environment interaction. *International journal of epidemiology.* 2003;32(1):38-48.

190. Delgado-Cruzata L, Wu HC, Liao Y, Santella RM, Terry MB. Differences in DNA methylation by extent of breast cancer family history in unaffected women. *Epigenetics : official journal of the DNA Methylation Society.* 2014;9(2):243-248.

191. Billroth T. Die allgemeine chirurgische Pathologie und Therapie in 51 Vorlesungen: ein Handbuch fur Studirende und Arzte, 14. Aufl. Berlin, Germany. G. Reimer. 1889; p. 908.

192. Carpenter CL, Jr., Derbes VJ, Jolly HW, Jr. Carcinoma of the Skin--a Guidepost to Internal Malignancy? *JAMA.* 1963;186:621-623.

193. Warren S, Gates O. Multiple Primary Tumors. *Am J Cancer.* 1932;16:1358-1414.

194. Liskow AS, Neugut AI, Benson M, Olsson CA, Birkhoff J, Chang CH. Multiple primary neoplasms in association with prostate cancer in black and white patients. *Cancer.* 1987;59(2):380-384.

195. Babacan NA, Aksoy S, Cetin B, et al. Multiple primary malignant neoplasms: multi-center results from Turkey. *J BUON.* 2012;17(4):770-775.

196. Vogt A, Schmid S, Heinimann K, et al. Multiple primary tumours: challenges and approaches, a review. *ESMO Open.* 2017;2(2):e000172.

197. Rebbeck TR, Mitra N, Wan F, et al. Association of type and location of BRCA1 and BRCA2 mutations with risk of breast and ovarian cancer. *JAMA.* 2015;313(13):1347-1361.

Appendix A: Supplemental Tables for Chapter 3

Supplemental Table 3.1. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping excluding cancer diagnoses prior to date of breast/ovarian cancer diagnosis for cases and date of interview for controls, Breast Cancer Family Registry

	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=80)**	**Multivariable model* (Cases n=2,072)**	**Multivariable model* (Cases n=198)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.39 (0.23, 0.62)	0.64 (0.56, 0.72)	0.35 (0.23, 0.45)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	2.37 (0.46, 7.93)	2.21 (1.74, 2.76)	1.53 (0.74, 2.86)
≥2 children	6.15 (3.01, 16.53)	2.30 (1.88, 2.73)	1.88 (1.12, 2.95)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.54 (0.33, 0.94)	0.80 (0.70, 0.92)	0.79 (0.55, 1.13)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.2. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping excluding proxies, Breast Cancer Family Registry

	DPBOC	BR	OV
Exposure	**Multivariable model* (Cases n=63)**	**Multivariable model* (Cases n=1,873)**	**Multivariable model* (Cases n=143)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.56 (0.32, 1.00)	0.74 (0.65, 0.84)	0.61 (0.39, 0.86)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	2.44 (0.37, 7.00)	2.31 (1.84, 2.94)	1.35 (0.63, 2.72)
≥2 children	4.47 (1.73, 11.31)	2.31 (1.89, 2.80)	1.39 (0.79, 2.41)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.56 (0.31, 1.11)	0.76 (0.66, 0.88)	0.82 (0.51, 1.25)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.3. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping excluding imputed menopausal status values, Breast Cancer Family Registry

	DPBOC	BR	OV
Exposure	**Multivariable model* (Cases n=61)**	**Multivariable model* (Cases n=1,799)**	**Multivariable model* (Case n=141)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.57 (0.33, 1.04)	0.77 (0.68, 0.87)	0.63 (0.41, 0.90)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	2.55 (0.40, 7.40)	2.37 (1.87, 3.05)	1.40 (0.66, 2.93)
≥2 children	4.39 (1.70, 11.14)	2.26 (1.84, 2.74)	1.39 (0.78, 2.43)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.53 (0.29, 1.06)	0.76 (0.66, 0.88)	0.83 (0.51, 1.26)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.4. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping excluding BOADICEA-imputed *BRCA1/2* mutation values, Breast Cancer Family Registry

***BRCA*1/2 Status Negative**			
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=20)**	**Multivariable model* (Cases n=1,305)**	**Multivariable model* (Cases n=74)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.54 (0.23, 1.41)	0.77 (0.66, 0.91)	0.45 (0.27, 0.72)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	2.68 (0.00, 20.24)	2.28 (1.72, 3.04)	1.10 (0.30, 2.68)
≥2 children	1.83 (0.30, 13.73)	2.03 (1.64, 2.57)	1.53 (0.81, 2.98)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.99 (0.30, 4.69)	0.75 (0.64, 0.90)	0.63 (0.34, 1.16)
***BRCA*1/2 Status Positive**			
	Multivariable model* (Cases n=35)	**Multivariable model* (Cases n=278)**	**Multivariable model* (Cases n=41)**
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.95 (0.43, 2.42)	0.99 (0.69, 1.50)	0.71 (0.29, 1.65)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	2.39 (0.00, 13.90)	2.52 (1.15, 5.47)	2.26 (0.40, 10.45)
≥2 children	7.19 (2.34, 42.92)	3.06 (1.54, 5.93)	1.40 (0.28, 6.10)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.39 (0.14, 1.08)	0.74 (0.51, 1.33)	1.15 (0.50, 4.26)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.5. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping excluding bilateral oophorectomies and mastectomies occurring prior to the date of breast/ovarian diagnosis for cases and date of interview for controls, Breast Cancer Family Registry

	DPBOC	BR	OV
Exposure	**Multivariable model* (Cases n=84)**	**Multivariable model* (Cases n=1,976)**	**Multivariable model* (Cases n=200)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.39 (0.23, 0.63)	0.65 (0.57, 0.74)	0.40 (0.27, 0.52)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	2.64 (0.57, 7.34)	2.33 (1.83, 2.94)	1.31 (0.61, 2.44)
≥2 children	6.00 (2.67, 14.24)	2.40 (1.95, 2.85)	1.74 (1.05, 2.75)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.54 (0.32, 0.91)	0.79 (0.69, 0.93)	0.84 (0.58, 1.23)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.6. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping including only confirmed cases, Breast Cancer Family Registry

	DPBOC**	BR	OV
Exposure	**Multivariable model* (Cases n=22)**	**Multivariable model* (Cases n=1,171)**	**Multivariable model* (Cases n=70)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.99 (0.35, 3.74)	0.86 (0.73, 1.00)	0.56 (0.34, 1.07)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	<0.01 (<0.01, 3.67)	2.21 (1.66, 2.89)	1.36 (0.35, 3.80)
≥2 children	10.80 (2.39, >999)	2.27 (1.77, 2.80)	1.76 (0.71, 3.82)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.39 (0.12, 1.42)	0.75 (0.64, 0.89)	1.04 (0.55, 1.91)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures
**Both cancers confirmed

Supplemental Table 3.7. Risk of developing both breast and ovarian cancer and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping including only epithelial ovarian cancer cases, Breast Cancer Family Registry

	DPBOC	OV
Exposure	**Multivariable model* (Cases n=30)**	**Multivariable model* (Cases n=70)**
	OR (95% CI)	OR (95% CI)
Oral contraceptive use		
Never	1.0	1.0
Ever	0.84 (0.32, 2.23)	0.54 (0.33, 1.02)
Parity		
Nulliparous	1.0	1.0
1 child	<0.01 (<0.01, <0.01)	1.38 (0.34, 4.12)
≥2 children	10.56 (2.79, 66.46)	1.68 (0.63, 3.82)
Breastfeed		
Never	1.0	1.0
Ever	0.34 (0.12, 0.88)	1.10 (0.56, 2.02)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.8. Risk of developing both breast and ovarian cancer and only breast cancer using unordered polytomous logistic regression with clustered bootstrapping excluding *in situ* breast cancers, Breast Cancer Family Registry

	DPBOC	BR
Exposure	**Multivariable model* (Cases n=84)**	**Multivariable model* (Cases n=2,008)**
	OR (95% CI)	OR (95% CI)
Oral contraceptive use		
Never	1.0	1.0
Ever	0.40 (0.23, 0.63)	0.62 (0.54, 0.69)
Parity		
Nulliparous	1.0	1.0
1 child	2.05 (0.39, 6.29)	2.38 (1.84, 3.01)
≥2 children	5.59 (2.68, 13.60)	2.49 (2.03, 2.96)
Breastfeed		
Never	1.0	1.0
Ever	0.51 (0.31, 0.90)	0.79 (0.69, 0.92)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.9. Risk of developing both breast and ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping excluding synchronous cases, Breast Cancer Family Registry

	DPBOC
Exposure	**Multivariable model* (Cases n=72)**
	OR (95% CI)
Oral contraceptive use	
Never	1.0
Ever	0.35 (0.19, 0.56)
Parity	
Nulliparous	1.0
1 child	3.35 (0.59, 13.92)
≥2 children	8.10 (3.60, 24.96)
Breastfeed	
Never	1.0
Ever	0.54 (0.31, 1.04)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.10. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping, Breast Cancer Family Registry

	BR-OV	OV-BR	DPBOC	BR	OV
Exposure	**Multivariable model*** **(Cases n=68)**	**Multivariable model*** **(Cases n=18)**	**Multivariable model*** **(Cases n=86)**	**Multivariable model*** **(Cases n=2,136)**	**Multivariable model*** **(Cases n=214)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Parity, Breastfeeding (BF)					
Nulliparous	1.0	1.0	1.0	1.0	1.0
Parous, Never BF	5.51 (2.53, 13.80)	3.03 (0.80, >999)	4.84 (2.40, 12.14)	2.37 (1.95, 2.80)	1.61 (0.99, 2.54)
Parous, Ever BF	2.79 (1.28, 6.81)	2.01 (0.86, >999)	2.59 (1.45, 5.83)	1.89 (1.61, 2.21)	1.44 (0.94, 2.16)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.11. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping stratified by 10-year risk of breast and ovarian cancer, Breast Cancer Family Registry

Breast/Ovarian Cancer Risk <20%			
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=46)**	**Multivariable model* (Cases n=1,759)**	**Multivariable model* (Cases n=157)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.24 (0.13, 0.50)	0.55 (0.49, 0.64)	0.34 (0.23, 0.50)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	1.73 (<0.01, 8.05)	2.08 (1.62, 2.73)	1.15 (0.46, 2.45)
≥2 children	3.69 (1.39, 13.32)	1.97 (1.68, 2.57)	1.43 (0.91, 2.57)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.88 (0.38, 1.68)	0.99 (0.75, 1.03)	1.09 (0.63, 1.38)
Breast/Ovarian Cancer Risk ≥20%			
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=35)**	**Multivariable model* (Cases n=275)**	**Multivariable model* (Cases n=44)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.60 (0.27, 1.54)	0.88 (0.61, 1.27)	0.39 (0.14, 0.66)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	5.19 (<0.01, 43.50)	2.98 (1.33, 5.67)	1.46 (0.16, 5.85)
≥2 children	11.42 (4.28, 84.53)	3.77 (1.97, 6.34)	1.23 (0.21, 4.09)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.20 (0.08, 0.54)	0.51 (0.35, 0.85)	0.88 (0.36, 4.48)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.12. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping stratified by tertiles of number of first and second degree relatives, Breast Cancer Family Registry

<14 First and Second Degree Relatives			
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=16)**	**Multivariable model* (Cases n=502)**	**Multivariable model* (Cases n=36)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.77 (0.30, 2.65)	1.03 (0.80, 1.31)	0.58 (0.25, 1.20)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	0.74 (<0.01, 5.42)	2.08 (1.41, 3.19)	1.51 (0.25, 5.56)
≥2 children	2.03 (0.41, 8.53)	1.79 (1.25, 2.64)	1.55 (0.43, 4.84)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.54 (0.11, 2.63)	0.84 (0.63, 1.19)	0.75 (0.27, 2.07)
14-20 First and Second Degree Relatives			
	Multivariable model* (Cases n=22)	**Multivariable model* (Cases n=681)**	**Multivariable model* (Cases n=47)**
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.53 (0.17, 1.64)	0.74 (0.59, 0.91)	0.80 (0.41, 1.87)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	10.71 (0.01, >999)	2.19 (1.42, 3.08)	0.79 (0.15, 2.31)
≥2 children	10.19 (2.07, >999)	1.81 (1.30, 2.41)	0.83 (0.27, 1.86)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.55 (0.18, 1.74)	0.74 (0.58, 0.97)	0.94 (0.50, 2.51)
>20 First and Second Degree Relatives			
	Multivariable model* (Cases n=25)	**Multivariable model* (Cases n=684)**	**Multivariable model* (Cases n=60)**
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.42 (0.16, 0.97)	0.53 (0.40, 0.64)	0.52 (0.24, 0.94)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	<0.01 (<0.01, 3.46)	2.10 (1.30, 3.76)	1.97 (0.49, 10.79)
≥2 children	4.26 (0.59, >999)	2.97 (1.96 4.58)	1.82 (0.82, 6.27)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.54 (0.17, 2.35)	0.67 (0.52, 0.85)	0.64 (0.33, 1.05)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.13. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping stratifying by center, Breast Cancer Family Registry

Philadelphia			
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=29)**	**Multivariable model* (Cases n=636)**	**Multivariable model* (Cases n=74)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.20 (0.07, 0.46)	0.47 (0.37, 0.59)	0.51 (0.29, 0.87)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	4.92 (0.71, 30.91)	2.14 (1.36, 3.45)	1.38 (0.36, 4.61)
≥2 children	3.90 (1.29, 21.21)	2.54 (1.79, 3.62)	2.25 (1.06, 5.71)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.36 (0.12, 0.88)	0.85 (0.67, 1.09)	0.57 (0.33, 1.01)
New York			
	Multivariable model* (Cases n=36)	**Multivariable model* (Cases n=1,202)**	**Multivariable model* (Cases n=100)**
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.42 (0.19, 0.86)	0.72 (0.62, 0.85)	0.26 (0.15, 0.40)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	0.59 (<0.01, 3.72)	2.13 (1.59, 2.77)	1.25 (0.43, 2.71)
≥2 children	3.74 (1.06, 17.91)	2.10 (1.66, 2.65)	1.13 (0.59, 2.27)
Breastfeed			
Never	1.0	1.0	1.0
Ever	1.06 (0.48, 2.80)	0.83 (0.70, 1.00)	1.31 (0.78, 2.33)
Utah			
	Multivariable model* (Cases n=21)	**Multivariable model* (Cases n=298)**	**Multivariable model* (Cases n=40)**
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.60 (0.15, 3.09)	0.54 (0.34, 0.70)	0.49 (0.15, 1.13)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	<0.01 (<0.01, 31.01)	3.92 (1.89, 9.59)	<0.01 (<0.01, 1.47)
≥2 children	25.44 (5.75, >999)	3.77 (1.80, 7.46)	3.78 (0.28, >999)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.18 (0.05, 0.81)	0.62 (0.39, 1.04)	0.70 (0.29, 4.48)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.14. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping by time since last parity, Breast Cancer Family Registry

	BR-OV	OV-BR	DPBOC	BR	OV
Exposure	**Multivariable model* (Cases n=67)**	**Multivariable model* (Cases n=18)**	**Multivariable model* (Cases n=85)**	**Multivariable model* (Cases n=2,121)**	**Multivariable model* (Cases n=213)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Time since last parity					
Nulliparous	0.29 (0.07, 1.03)	0.39 (<0.01, 1.83)	0.31 (0.09, 0.86)	0.63 (0.52, 0.81)	0.50 (0.29, 0.97)
<10 years	1.02 (0.32, 3.53)	0.55 (<0.01, 4.02)	0.92 (0.32, 2.71)	1.50 (1.20, 1.92)	0.58 (0.32, 1.14)
10 to <20 years	3.48 (1.39, 8.36)	2.64 (0.55, 12.05)	3.28 (1.46, 6.93)	2.23 (1.81, 2.69)	0.92 (0.56, 2.55)
20+ years	1.0	1.0	1.0	1.0	1.0

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.15. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping by time between menarche and first parity, Breast Cancer Family Registry

	BR-OV	OV-BR	DPBOC	BR	OV
Exposure	**Multivariable model* (Cases n=53)**	**Multivariable model* (Cases n=11)**	**Multivariable model* (Cases n=64)**	**Multivariable model* (Cases n=1,875)**	**Multivariable model* (Cases n=149)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Time between menarche and first parity					
Nulliparous	0.30 (0.10, 0.73)	0.67 (<0.01, 9.89)	0.35 (0.13, 0.77)	0.45 (0.37, 0.54)	0.91 (0.52, 1.55)
<10 years	1.03 (0.40, 1.53)	1.50 (0.25, 7.82)	1.10 (0.48, 1.60)	0.91 (0.75, 1.01)	1.24 (0.73, 1.76)
10 to <20 years	1.0	1.0	1.0	1.0	1.0
20+ years	0.29 (<0.01, 1.06)	<0.01 (<0.01, <0.01)	0.26 (<0.01, 0.93)	0.98 (0.76, 1.20)	0.80 (0.28, 1.49)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.16. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping stratified birth cohort, Breast Cancer Family Registry

	Born before 1930 (aged ≥45 in 1975)		
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=31)**	**Multivariable model* (Cases n=474)**	**Multivariable model* (Cases n=66)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	1.26 (0.23, 4.42)	0.87 (0.50, 1.51)	0.25 (<0.01, 0.80)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	1.40 (<0.01, >999)	1.34 (0.56, 3.28)	0.74 (<0.01, 4.95)
≥2 children	2.82 (0.63, >999)	0.99 (0.48, 1.97)	1.02 (0.34, 6.00)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.80 (0.26, 2.28)	1.46 (1.01, 2.12)	1.53 (0.81, 3.27)
	Born between 1930 and 1960 (aged 15 to 45 in 1975)		
	Multivariable model* (Cases n=52)	**Multivariable model* (Cases n=1,365)**	**Multivariable model* (Cases n=126)**
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.69 (0.36, 1.45)	0.73 (0.61, 0.86)	0.53 (0.35, 0.77)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	1.93 (0.30, 8.35)	1.77 (1.32, 2.35)	0.98 (0.41, 2.06)
≥2 children	3.37 (1.51, 10.62)	1.82 (1.41, 2.26)	1.14 (0.64, 1.93)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.39 (0.21, 0.79)	0.61 (0.51, 0.73)	0.72 (0.47, 1.15)
	Born after 1960 (≤15 in 1975)		
	Multivariable model* (Cases n=3)	**Multivariable model* (Cases n=297)**	**Multivariable model* (Cases n=22)**
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.06 (<0.01, >999)	0.91 (0.63, 1.24)	0.23 (0.07, 0.51)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	<0.01 (<0.01, 3.12)	1.81 (0.96, 2.91)	1.66 (<0.01, 7.61)
≥2 children	<0.01 (<0.01, <0.01)	1.44 (0.81, 2.15)	1.56 (0.09, 7.32)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.50 (<0.01, 902.96)	0.83 (0.57, 1.44)	0.42 (0.08, 2.71)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.17. Risk of developing both breast and ovarian cancer compared to only breast cancer or only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping stratified birth cohort, Breast Cancer Family Registry

	BR-OV vs. BR	BR-OV vs. OV	OV-BR vs. OV	OV-BR vs. BR
Exposure	**Multivariable model* (Cases n=68)**	**Multivariable model* (Cases n=68)**	**Multivariable model* (Cases n=18)**	**Multivariable model* (Cases n=18)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use				
Never	1.0	1.0	1.0	1.0
Ever	0.63 (0.36, 1.10)	1.08 (0.57, 2.17)	0.80 (0.16, 2.04)	0.47 (0.09, 1.14)
Parity				
Nulliparous	1.0	1.0	1.0	1.0
1 child	1.50 (0.33, 4.39)	2.69 (0.52, 10.05)	<0.01 (<0.01, 3.19)	<0.01 (<0.01, 1.66)
≥2 children	2.68 (1.19, 6.73)	3.78 (1.53, 10.38)	2.34 (0.64, >999)	1.66 (0.45, >999)
Breastfeed				
Never	1.0	1.0	1.0	1.0
Ever	0.61 (0.35, 1.06)	0.55 (0.28, 1.08)	0.71 (0.23, 3.36)	0.79 (0.25, 3.45)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.18. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping in parous women by duration of OC use pre- and post-first parity, Breast Cancer Family Registry

OC use pre-pregnancy only			
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=46)**	**Multivariable model* (Cases n=1,191)**	**Multivariable model* (Cases n=131)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Duration of OC use			
Never Use	1.0	1.0	1.0
<5 years	0.24 (0.06, 0.48)	0.50 (0.41, 0.61)	0.28 (0.13, 0.47)
≥5 years	0.07 (<0.01, 0.25)	0.42 (0.31, 0.58)	0.23 (0.04, 0.44)
OC use post-pregnancy			
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=58)**	**Multivariable model* (Cases n=1,360)**	**Multivariable model* (Cases n=147)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Duration of OC use			
Never Use	1.0	1.0	1.0
<5 years	0.33 (0.11, 0.64)	0.51 (0.40, 0.60)	0.25 (0.11, 0.43)
≥5 years	0.38 (0.17, 0.74)	0.50 (0.40, 0.59)	0.35 (0.18, 0.52)

*Adjusted for age, breastfeeding, menopausal status, alcohol consumption, and BCFR site

Supplemental Table 3.19. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping in women who have used oral contraceptives by duration of use, Breast Cancer Family Registry

OC use ≥ 5 years			
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=15)**	**Multivariable model* (Cases n=482)**	**Multivariable model* (Cases n=32)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	3.24 (<0.01, >999)	2.23 (1.45, 3.33)	2.31 (0.38, 19.03)
≥2 children	3.63 (1.05, >999)	1.80 (1.21, 2.55)	2.07 (0.51, 14.87)
OC use < 5 years			
	DPBOC	**BR**	**OV**
	Multivariable model* (Cases n=17)	**Multivariable model* (Cases n=585)**	**Multivariable model* (Cases n=39)**
Parity			
Nulliparous	1.0	1.0	1.0
1 child	2.67 (<0.01, >999)	2.46 (1.63, 3.86)	0.65 (<0.01, 2.95)
≥2 children	3.37 (<0.01, >999)	3.06 (2.06, 4.30)	0.80 (0.23, 2.73)

*Adjusted for age, breastfeeding, menopausal status, alcohol consumption, and BCFR site

Supplemental Table 3.20. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping stratified by treatment, Breast Cancer Family Registry

Any chemotherapy, radiotherapy, hormone therapy			
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=34)**	**Multivariable model* (Cases n=1,252)**	**Multivariable model* (Cases n=75)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.51 (0.25, 1.08)	0.87 (0.74, 1.00)	0.55 (0.30, 0.89)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	1.00 (<0.01, 8.44)	2.44 (1.86, 3.19)	1.21 (0.32, 3.29)
≥2 children	5.18 (1.41, 43.57)	2.27 (1.80, 2.85)	1.69 (0.75, 3.35)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.78 (0.33, 2.39)	0.72 (0.61, 0.86)	0.82 (0.45, 1.44)
Surgery only			
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=24)**	**Multivariable model* (Cases n=523)**	**Multivariable model* (Cases n=32)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.31 (0.10, 0.82)	0.45 (0.36, 0.53)	0.25 (0.09, 0.56)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	1.23 (<0.01, 9.84)	2.13 (1.36, 3.21)	0.83 (<0.01, 3.59)
≥2 children	4.71 (0.86, 49.97)	3.20 (2.14, 4.58)	0.91 (0.19, 2.75)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.91 (0.31, 3.26)	1.00 (0.78, 1.29)	1.18 (0.51, 4.95)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

Supplemental Table 3.21. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping stratified by time since diagnosis, Breast Cancer Family Registry

Time Between Diagnosis and Interview ≤2 years**			
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=4)**	**Multivariable model* (Cases n=735)**	**Multivariable model* (Cases n=71)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.31 (0.04, 2.29)	1.10 (0.91, 1.32)	1.20 (0.70, 2.06)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	2.43 (0.09, 64.26)	1.44 (1.05, 1.97)	1.24 (0.48, 3.18)
≥2 children	0.90 (0.04, 20.32)	1.33 (1.02, 1.74)	1.14 (0.53, 2.43)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.73 (0.06, 8.73)	0.98 (0.79, 1.20)	0.83 (0.46, 1.49)
Time Between Diagnosis and Interview >2 years**			
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=59)**	**Multivariable model* (Cases n=1,138)**	**Multivariable model* (Cases n=72)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.56 (0.32, 0.98)	0.58 (0.50, 0.67)	0.31 (0.18, 0.52)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	2.39 (0.68, 8.45)	3.29 (2.49, 4.36)	1.51 (0.56, 4.08)
≥2 children	5.10 (2.05, 12.68)	3.38 (2.68, 4.28)	1.72 (0.80, 3.70)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.55 (0.30, 1.00)	0.65 (0.55, 0.77)	0.85 (0.48, 1.52)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

**Non-proxies only

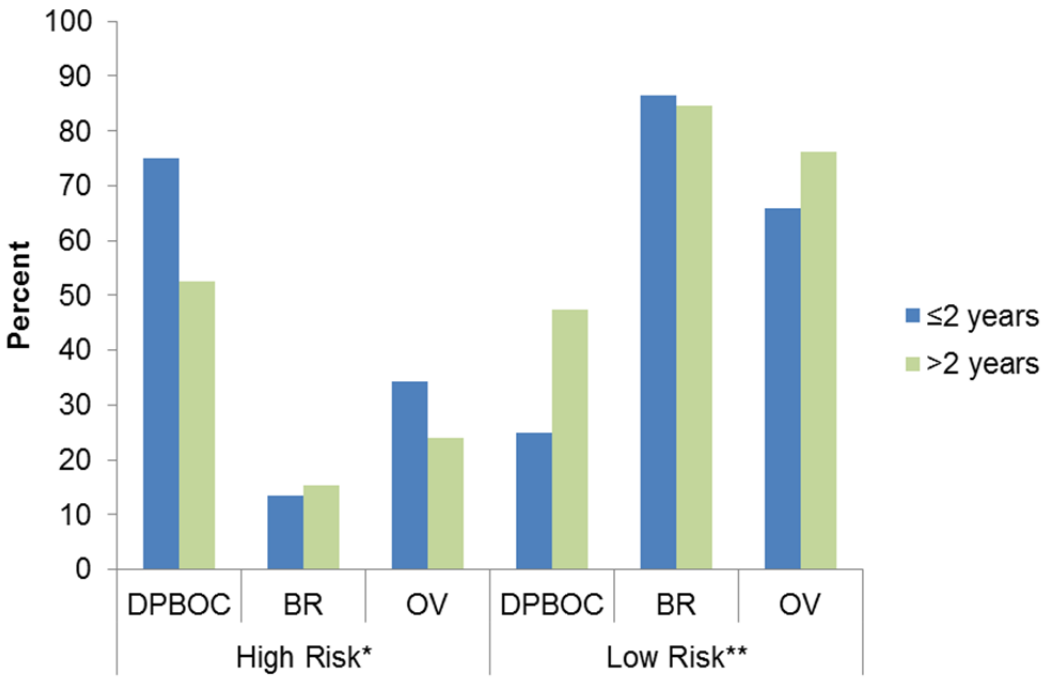
Supplemental Table 3.22. Risk of developing both breast and ovarian cancer, only breast cancer, and only ovarian cancer using unordered polytomous logistic regression with clustered bootstrapping stratified by time since diagnosis, Breast Cancer Family Registry

Time Between Diagnosis and Interview ≤5 years**			
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=12)**	**Multivariable model* (Cases n=1,098)**	**Multivariable model* (Cases n=99)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.97 (0.24, 7.25)	0.99 (0.84, 1.16)	0.80 (0.50, 1.33)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	0.98 (<0.01, 6.43)	1.87 (1.40, 2.45)	1.10 (0.40, 2.51)
≥2 children	1.14 (<0.01, 10.27)	1.55 (1.20, 1.93)	1.10 (0.54, 2.17)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.53 (0.07, >999)	0.93 (0.78, 1.11)	0.89 (0.50, 1.52)
Time Between Diagnosis and Interview >5 years**			
	DPBOC	**BR**	**OV**
Exposure	**Multivariable model* (Cases n=51)**	**Multivariable model* (Cases n=775)**	**Multivariable model* (Cases n=44)**
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Oral contraceptive use			
Never	1.0	1.0	1.0
Ever	0.47 (0.25, 0.84)	0.49 (0.40, 0.58)	0.31 (0.13, 0.51)
Parity			
Nulliparous	1.0	1.0	1.0
1 child	3.65 (<0.01, 18.27)	3.10 (2.27, 4.73)	2.28 (0.42, 9.19)
≥2 children	7.50 (2.46, 31.60)	4.22 (3.12, 5.79)	2.49 (0.89, 8.54)
Breastfeed			
Never	1.0	1.0	1.0
Ever	0.55 (0.27, 1.22)	0.58 (0.47, 0.71)	0.76 (0.35, 1.56)

*Adjusted for age, menopausal status, alcohol consumption, BCFR site, and other main exposures

**Non-proxies only

Supplemental Figure 3.1. Percent of both breast and ovarian cancer cases, only breast cancer cases, and only ovarian cancer cases at high risk versus low risk of breast or ovarian cancer by time since diagnosis, Breast Cancer Family Registry



*High risk defined as having a 10-year breast or ovarian cancer risk greater than or equal to 20%
**Low risk defined as having a 10-year breast or ovarian cancer risk less than 20%

Supplemental Table 3.23. Number of cases per group for Table 2

	BR-OV (n=68)	OV-BR (n=18)	DPBOC (n=86)	BR (n=2,136)	OV (n=214)
Exposure	**N Cases**	**N Cases**	**N Cases**	**N Cases**	**N Cases**
Oral contraceptive use					
Never	34	12	46	996	135
Ever	34	6	40	1,140	79
Parity					
Nulliparous	10	3	13	374	39
1 child	6	0	6	267	18
≥2 children	52	15	67	1,495	157
Age at First Birth					
Nulliparous	10	3	13	373	39
<20 years	8	1	9	206	20
20-24 years	34	10	44	699	75
25-29 years	12	3	15	548	57
>29 years	4	1	5	304	22
Age at Last Birth					
Nulliparous	10	3	13	374	39
<25 years	9	1	10	212	15
25-29 years	22	4	26	572	54
30-34 years	18	7	25	582	56
>34 years	8	3	11	381	49
Breastfeed					
Never	35	9	44	1,010	99
Ever	33	9	42	1,126	115

Appendix B: Supplemental Tables for Chapter 4

Supplemental Table 4.1. Hormonal and reproductive factors and risk of BR-OV, OV, and BR using Cox proportional hazards with baseline variables, ProF-SC

	BR-OV		OV		BR	
	Model 1*	**Model 2****	**Model 1***	**Model 2****	**Model 1***	**Model 2****
Exposure	**Events (n = 68) Person-time*** (111,152 py)**	**Events (n = 68) Person-time*** (111,152 py)**	**Events (n = 68) Person-time*** (133,712 py)**	**Events (n = 68) Person-time*** (133,712 py)**	**Events (n = 748) Person-time*** (145,052 py)**	**Events (n = 748) Person-time*** (145,052 py)**
	HR (95% CI)	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Oral Contraceptive Use						
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Ever	1.31 (0.74, 2.32)	1.58 (0.89, 2.83)	0.64 (0.38, 1.06)	0.56 (0.34, 0.94)	1.18 (0.98, 1.42)	1.14 (0.94, 1.37)
Number of FT Pregnancies						
Nulliparous	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
1	0.78 (0.32, 1.88)	0.80 (0.33, 1.97)	0.11 (0.01, 0.84)	0.12 (0.02, 0.96)	0.90 (0.67, 1.20)	0.99 (0.74, 1.35)
≥2	0.65 (0.32, 1.33)	0.60 (0.29, 1.23)	0.83 (0.38, 1.79)	1.03 (0.48, 2.22)	0.80 (0.63, 1.01)	0.90 (0.70, 1.16)
Breastfeeding						
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Ever	1.26 (0.70, 2.28)	1.26 (0.71, 2.24)	1.14 (0.62, 2.09)	1.12 (0.60, 2.10)	1.10 (0.92, 1.32)	1.06 (0.88, 1.27)

*Regression models conditional on birth cohort; hazard ratios include all main exposures (OC use, number of FT pregnancies, and breastfeeding)

**Regression models conditional on birth cohort; hazard ratios adjusted for study center, race/ethnicity, age at menarche, menopausal status, BMI, height, education, cigarette use, alcohol consumption, hormone replacement therapy, and all main exposures

***Age is the time-scale

Supplemental Table 4.2. Hormonal and reproductive factors and risk of BR-OV using Cox proportional hazards with variables truncated to the age at diagnosis of the first primary breast cancer, ProF-SC

	BR-OV	
	Model 1*	**Model 2****
Exposure	**Events (n = 68) Person-time*** (111,152 py)**	**Events (n = 68) Person-time*** (111,152 py)**
	HR (95% CI)	**HR (95% CI)**
Oral Contraceptive Use		
Never	1.00 (Ref)	1.00 (Ref)
Ever	1.32 (0.75, 2.33)	1.57 (0.88, 2.80)
Number of FT Pregnancies		
Nulliparous	1.00 (Ref)	1.00 (Ref)
1	0.84 (0.36, 1.98)	0.88 (0.37, 2.11)
≥2	0.63 (0.31, 1.30)	0.59 (0.29, 1.21)
Breastfeeding		
Never	1.00 (Ref)	1.00 (Ref)
Ever	1.27 (0.70, 2.30)	1.25 (0.70, 2.23)

*Regression models conditional on birth cohort; hazard ratios include all main exposures (OC use, number of FT pregnancies, and breastfeeding)

**Regression models conditional on birth cohort; hazard ratios adjusted for study center, race/ethnicity, age at menarche, menopausal status, BMI, height, education, cigarette use, alcohol consumption, hormone replacement therapy, and all main exposures

***Age is the time-scale

Supplemental Table 4.3. Hormonal and reproductive factors and risk of BR-OV and BR using Cox proportional hazards excluding *in situ* breast cancers, ProF-SC

	BR-OV	BR
	Multivariable Model*	Multivariable Model*
Exposure	Events (n = 64) Person-time** (103,539 py)	Events (n = 646) Person-time** (145,052 py)
	HR (95% CI)	HR (95% CI)
Oral Contraceptive Use		
Never	1.00 (Ref)	1.00 (Ref)
Ever	1.51 (0.84, 2.72)	1.17 (0.96, 1.43)
Number of FT Pregnancies		
Nulliparous	1.00 (Ref)	1.00 (Ref)
1 Child	0.59 (0.23, 1.56)	0.96 (0.68, 1.35)
≥2 Children	0.45 (0.21, 0.94)	0.96 (0.73, 1.26)
Breastfeeding		
Never	1.00 (Ref)	1.00 (Ref)
Ever	1.40 (0.74, 2.68)	1.01 (0.82, 1.24)

*Regression models conditional on birth cohort; hazard ratios adjusted for study center, race/ethnicity, age at menarche, menopausal status, BMI, height, education, cigarette use, alcohol consumption, hormone replacement therapy, and all main exposures

**Age is the time-scale

Supplemental Table 4.4. Comparison of Cox proportional hazards and Poisson regression results, ProF-SC

	BR-OV		OV		BR	
	Cox PH	Poisson	Cox PH	Poisson	Cox PH	Poisson
	Multivariable Model*	Multivariable Model*	Multivariable Model*	Multivariable Model*	Multivariable Model*	Multivariable Model*
Exposure	Events (n = 68) Person-time** (111,152 py)	Events (n = 68) Person-time** (111,152 py)	Events (n = 68) Person-time** (133,712 py)	Events (n = 68) Person-time** (133,712 py)	Events (n = 748) Person-time** (145,052 py)	Events (n = 748) Person-time** (145,052 py)
	HR (95% CI)	RR (95% CI)	HR (95% CI)	RR (95% CI)	HR (95% CI)	RR (95% CI)
Oral Contraceptive Use						
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Ever	1.62 (0.91, 2.90)	1.53 (0.85, 2.74)	0.59 (0.34, 1.00)	0.56 (0.15, 2.14)	1.14 (0.95, 1.38)	1.04 (0.54, 2.02)
Number of FT Pregnancies						
Nulliparous	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
1	0.64 (0.26, 1.62)	0.65 (0.26, 1.62)	0.56 (0.17, 1.81)	0.68 (0.03, 15.15)	0.98 (0.72, 1.32)	1.23 (0.40, 3.77)
≥2	0.47 (0.22, 0.97)	0.49 (0.22, 1.06)	1.02 (0.44, 2.38)	1.33 (0.16, 10.99)	0.88 (0.68, 1.13)	1.21 (0.47, 3.11)
Breastfeeding						
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Ever	1.37 (0.74, 2.56)	1.38 (0.72, 2.65)	1.05 (0.56, 1.98)	0.98 (0.19, 5.01)	1.05 (0.86, 1.27)	0.98 (0.48, 2.01)

*Regression models conditional on birth cohort; hazard ratios adjusted for study center, race/ethnicity, age at menarche, menopausal status, BMI, height, education, cigarette use, alcohol consumption, hormone replacement therapy, and all main exposures

**Age is the time-scale

Supplemental Table 4.5. Hormonal and reproductive factors and risk of BR-OV, OV, and BR by *BRCA*1/2 status, ProF-SC

***BRCA*1/2 Positive**			
	BR-OV	**OV**	**BR**
Exposure	**Multivariable Model***	**Multivariable Model****	**Multivariable Model***
	Events (n = 23) Person-time (6,550 py)**	**Events (n = 17) Person-time** (4,188 py)**	**Events (n = 101) Person-time** (5,457 py)**
	HR (95% CI)	**HR (95% CI)**	**HR (95% CI)**
Oral Contraceptive Use			
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Ever	2.10 (0.80, 5.48)	-	1.23 (0.68, 2.22)
Number of Full-Term Pregnancy			
Nulliparous	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
1	0.50 (0.07, 3.86)	-	2.66 (1.19, 5.91)
≥2	0.25 (0.05, 1.19)	-	1.17 (0.65, 2.14)
Breastfeeding			
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Ever	3.12 (0.82, 11.80)	-	0.68 (0.39, 1.18)
***BRCA*1/2 Negative**			
	Events (n = 45) Person-time (104,602 py)**	**Events (n = 51) Person-time** (129,524 py)**	**Events (n = 647) Person-time** (139,595 py)**
	HR (95% CI)	**HR (95% CI)**	**HR (95% CI)**
Oral Contraceptive Use			
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Ever	1.33 (0.66, 2.70)	0.79 (0.43, 1.45)	1.16 (0.95, 1.42)
Number of Full-Term Pregnancy			
Nulliparous	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
1	0.60 (0.20, 1.75)	0.28 (0.06, 1.31)	0.92 (0.66, 1.27)
≥2	0.48 (0.20, 1.12)	0.70 (0.27, 1.79)	0.84 (0.64, 1.10)
Breastfeeding			
Never	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Ever	1.02 (0.48, 2.15)	1.30 (0.59, 2.87)	1.06 (0.87, 1.30)

*Regression models conditional on birth cohort; hazard ratios adjusted for study center, race/ethnicity, age at menarche, menopausal status, BMI, height, education, cigarette use, alcohol consumption, hormone replacement therapy, and all main exposures

**Age is the time-scale

Supplemental Table 4.6. Hormonal and reproductive factors and risk of BR-OV stratified by time between breast cancer diagnosis and baseline interview, ProF-SC

Time Between Diagnosis and Interview ≤2 years*	
	BR-OV
Exposure	**Multivariable model***
	(Events n=40) **Person-time** (60,084 py)**
	HR (95% CI)
Oral contraceptive use	
Never	1.0
Ever	1.07 (0.51, 2.25)
Number of FT Pregnancies	
Nulliparous	1.0
1	0.85 (0.26, 2.80)
≥2	0.46 (0.17, 1.25)
Breastfeeding	
Never	1.0
Ever	1.43 (0.59, 3.48)
Time Between Diagnosis and Interview >2 years*	
	BR-OV
Exposure	**Multivariable model***
	(Events n=28) **Person-time** (51,068 py)**
	HR (95% CI)
Oral contraceptive use	
Never	1.0
Ever	2.82 (1.16, 6.88)
Number of FT Pregnancies	
Nulliparous	1.0
1	0.34 (0.06, 1.88)
≥2	0.48 (0.16, 1.40)
Breastfeed	
Never	1.0
Ever	1.38 (0.55, 3.51)

*Regression models conditional on birth cohort; hazard ratios adjusted for study center, race/ethnicity, age at menarche, menopausal status, BMI, height, education, cigarette use, alcohol consumption, hormone replacement therapy, and all main exposures

**Age is the time-scale